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CONTENTS OF THIS ISSUE

Heat Denatured Gelatin 631
L. F. Tice and *A. W. Moore*

Solubility of Iodine in Glycol-Water Solutions 634
Arthur Osol and *Charles C. Pines*

Potential Ergot Substitutes. Esters and Amides of *beta*-Amino Acids 637
P. E. Norris and *F. F. Blicke*

The Identification and Determination of N-Allylnormorphine Hydrochloride 640
W. J. Seagers, J. D. Neuss, and *W. J. Mader*

Isoquinoline Derivatives As Local Anesthetics 643
Elvin L. Anderson, James W. Wilson, and *Glenn E. Ullyot*

A Comparative Study of Prantal and Banthine in Hyperhidrosis 651
Arthur G. Zupko and *Leon D. Prokop*

The Effects of Surface-Active Agents on Bacitracin Activity *in Vitro* 654
C. B. Bruce and *L. Mitchell*

Routes to the Synthesis of 6-Amino-2-Naphthoic Acid 657
G. Stapleton and *A. I. White*

The Effect of Phenergan on Tetanus Spore Infection 658
Louis Gershenfeld, Joseph W. E. Harrisson, and *Arthur E. Greene*

Extraction of Belladonna Root by Various Furans 660
E. D. Carkhuff and *L. G. Gramling*

Acute and Short-Term Oral Toxicity Tests of Ferric Dimethyldithiocarbamate (Ferbam) and Zinc Dimethyldithiocarbamate (Ziram) 662
Harold C. Hodge, Elliott A. Maynard, William Downs, Harvey J. Blanchet, Jr., and *Chester K. Jones*

Studies on Species of *Asclepias*. VII. Determination of Palisade Ratio 666
W. E. Hassan, Jr., R. A. Gosselin, R. W. Vander Wyk, and *M. W. Quimby*

Studies on Species of *Asclepias*. VIII. Determination of Vein Islet Numbers 668
W. E. Hassan, Jr., R. A. Gosselin, R. W. Vander Wyk, and *M. W. Quimby*

The Spectrophotometric and Polarographic Determination of Isonicotinic Acid Hydrazide 670
J. D. Neuss, W. J. Seagers, and *W. J. Mader*

Composition of Gum Turpentines of Pines. XIV. A Report on Three Mexican Pines: *Pinus ayacahuite, Pinus cembroides,* and *Pinus pinceana* 673
N. T. Mirov

Composition of Gum Turpentines of Pines. XV. A Report on *Pinus resinosa* and *Pinus reflexa* 677
N. T. Mirov

A Note on the Preparation of *l*-Menthyl Acetate 679
Clifton E. Miller

Book Notices 680

Index to Journal for 1952 681

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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

VOLUME XLI | *DECEMBER, 1952* | NUMBER 12 CONSECUTIVE NO. 24

Heat Denatured Gelatin*

By L. F. TICE and A. W. MOORE

Gelatin, when subjected to prolonged heating at high temperatures, is denatured. Such heat-denatured gelatin is altered in its solubility characteristics but is still subject to enzymatic hydrolysis. The changes which take place in gelatin thus treated are described, and certain applications of the product are suggested.

VERY FEW references appear in the literature concerning the heat denaturation of gelatin. Gombay (1) discussed the dehydration of gelatin both by vacuum drying and by heating; he described two kinds of water removed, capillary water and colloid water, and stated that the vacuum dehydration is reversible whereas heat dehydration is not. Lipatov and Lebedeva (2) heated gelatin at 110–130° for periods ranging from thirty minutes to twelve hours and reported a number of changes, including an increase in swelling and reduced solubility. Correll (3), in a patent, describes the preparation of a surgical gelatin dusting powder by heating dry gelatin or gelatin previously treated with formaldehyde until it is insoluble in water but remains digestible by a proteolytic enzyme, namely, pepsin. The language of the patent is quite broad as are the conditions described for denaturing the gelatin.

The work reported in this paper was undertaken to determine more precisely the temperature/time relationships in denaturing gelatin by dry heat and also to study the progressive changes which take place as gelatin is heated at different temperature levels over long periods of time.

EXPERIMENTAL

Materials.—Two samples of gelatin were used for this study; one, Pharmagel A, was derived from an acid-treated precursor (porkskins) and the other, Pharmagel B, was derived from an alkali-treated precursor (ossein). The specifications of the gelatins used are listed in Table I.

TABLE I

	Pharmagel A	Pharmagel B
Bloom strength	227	212
Viscosity (C. P. S.)	41	56.1
*p*H	4.2	6.2
Raw material	Porkskin	Bone
Ash	Less than 0.4%	1%

Procedure.—Weighed samples of both gelatins were placed in porcelain evaporating dishes and heated in electric ovens at 4 temperature levels: 120, 150, 170, and 190°. It was soon evident that excessive carbonization took place at 190°; thus this series was abandoned. Representative samples were removed at intervals of one, two, four, eight, and sixteen days, placed in tightly closed bottles, and reserved for subsequent study.

An attempt was made to heat gelatin in sealed glass ampuls, but the ampuls burst from the development of internal pressure, as might be expected.

* Received August 22, 1952, from the Philadelphia College of Pharmacy and Science, Philadelphia, Pa.
Presented to the Scientific Section, A. PH. A., Philadelphia meeting, August, 1952.

In order to determine whether dehydration *per se* was responsible for the effect of heat on gelatin, samples of the test gelatin were placed in desiccators over magnesium perchlorate (Dehydrite) under both normal and reduced pressure (10 mm.) for twenty-five days. All samples were examined for weight loss, degree of swelling in cold water, change in solubility, and degree of digestion, using both pepsin and papain.

RESULTS

Weight Loss.—While all samples showed a progressive loss in weight during heating, the results were inconsistent and were not such that they could be presented in graph form. No explanation for this is offered, since there were a number of possible variables in both samples and technique.

It was of interest to note that under vacuum desiccation, the weight loss was of about the same magnitude as that caused by prolonged heating. No important change in properties occurred, however, through loss of water by vacuum desiccation as was the case following heat treatment. This would seem to indicate that heat causes changes in the gelatin other than dehydration. Since heat is known to effect polymerization of other proteins, it is reasonable to assume that such takes place in gelatin.

Color and Solubility.—In each of the samples heated, there was a gradual increase in color. The higher the temperature, the more rapid was the development of color through yellow to brown. Much of this colored material could be extracted with water; the extracted material had an unpleasant taste.

Solubility was determined as follows: one gram of the sample was mixed with 19 cc. of cold water, and the mixture was then heated at 95° for one hour with occasional stirring. The mixture was filtered through a Buchner funnel, and the residue was washed with hot water, dried, and weighed. Figure 1 shows the results obtained using Pharmagel A; Fig. 2 shows the results using Pharmagel B. From these, it will be seen that heating at 170° produces about the same effect in a relatively short time as heating at 150° for a longer time. At the higher temperature used, considerable color was produced over a long period and there was evidence of decomposition since the soluble fraction increased with increased time of heating. This was most pronounced in samples heated at 170°, using Pharmagel B. A very interesting observation was made concerning samples heated for only a short time at a temperature of 120°. These, while not soluble in water, gave a very thick hydrogel not unlike that given by tragacanth. These hydrogels were still quite viscous at high temperatures, unlike an ordinary gelatin gel.

Degree of Swelling.—The degree of swelling produced by absorption of water was determined by placing 1 Gm. of the sample in 19 cc. of water at 5° and maintaining it at 5° for eighteen hours. The volume occupied by the swollen gelatin as compared with its original volume was taken as the index of swelling.

Heating at all temperature levels progressively reduced the capacity of the gelatin to absorb water and swell. All samples, in time, reached an equilibrium in which their capacity to absorb water was reduced to about 25% of the original, in the case of Pharmagel A, and 50% of the original, in the case of Pharmagel B. This is illustrated in Figs. 3 and 4.

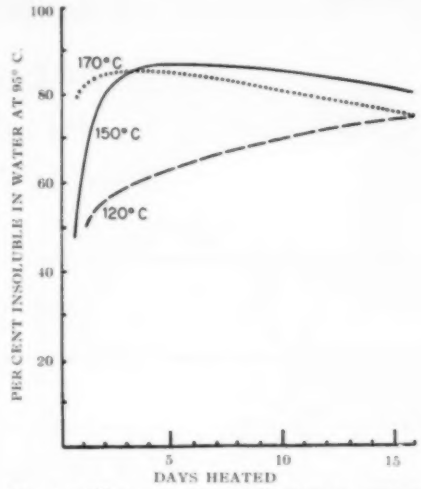

Fig. 1.—Effect of prolonged heating at various temperatures on the solubility of Pharmagel A in hot water.

Proteolytic Digestion.—The effect of both pepsin and papain on the heat-treated samples was determined. The method used for pepsin was as follows: 5% (w/v) of the sample was added to a solution containing 0.1 *N* hydrochloric acid and 1% pepsin N. F. (Merck & Co.). The mixture was then incu-

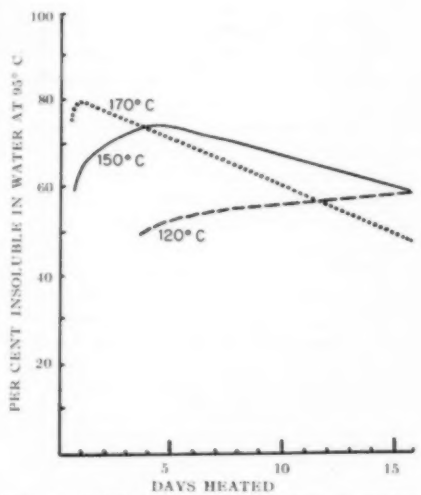

Fig. 2.—Effect of prolonged heating at various temperatures on the solubility of Pharmagel B in hot water.

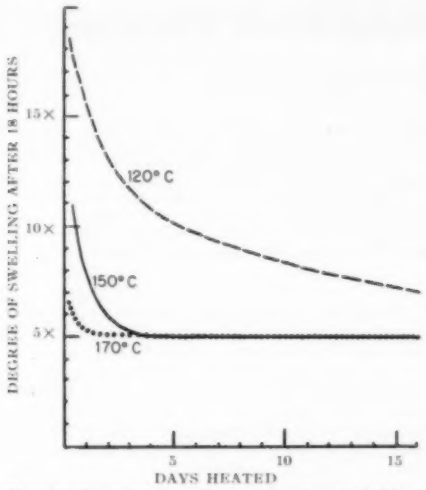

Fig. 3.—Degree of swelling of heat-treated Pharmagel A when added as a 5% suspension to water and maintained at 5°C.

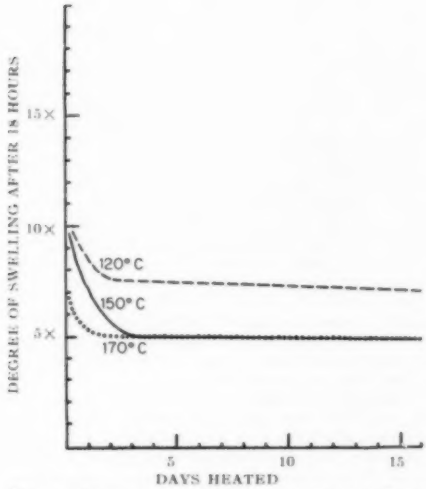

Fig. 4.—Degree of swelling of heat-treated Pharmagel B when added as a 5% suspension to water and maintained at 5°C.

bated at approximately 37° for eighteen hours. A control, using 0.1 *N* hydrochloric acid without pepsin, was run in each case. The ratio of clear supernatant liquid in the digestion tube over that in the control was taken as the index of digestion. The method using papain (Merck & Co.) was similar except that no hydrochloric acid was used and the control was simply distilled water.

Gelatin heated at 120°, even for as long as sixteen days, was still completely digested by both pepsin and papain. Gelatin heated at 150° for four days was not digested by pepsin although completely digested by papain. After eight days at 150°, there was a small fraction not digested by papain in the case of the heat-treated Pharmagel A. When heated as little as one day at 170°, little of the gelatin was digested by pepsin although papain completely digested the sample. After two days at 170°, papain was able to digest all but a small quantity of the Pharmagel A. At the end of sixteen days, papain still digested most of the sample. Throughout all experiments, Pharmagel B seemed more readily digested by papain than was Pharmagel A. Whether this was due to a difference in *p*H was not determined, but the B is a more nearly neutral product. Pepsin, throughout all experiments, seemed much less effective than papain.

As in most enzymatic digestion experiments, there were a few inconsistent results which are not considered significant.

The water control in the case of papain and the 0.1% hydrochloric acid control in the case of pepsin made it possible to show where enzymatic digestion was taking place, rather than simple solution or ordinary hydrolysis.

SUMMARY

1. Gelatins from both an acid-treated precursor (Pharmagel A) and an alkali-treated precursor (Pharmagel B) were subjected to prolonged dry heat at temperatures of 120, 150, and 170°. Similar samples were dehydrated by vacuum desiccation over magnesium perchlorate.

2. The changes produced in solubility, degree of swelling in water, and digestibility with both pepsin and papain were determined.

3. The results obtained show that denaturation occurs at all of the temperatures employed. A shorter time is required with higher temperatures.

4. Evidence is presented showing that the denaturation is not caused by dehydration *per se*.

5. In denaturing gelatin, the product passes through a progressive change from altered solubility to that state in which even digestive enzymes are not able to hydrolyze it completely.

6. Papain seems a more effective enzyme than pepsin in digesting denatured gelatin.

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(2) Lipatov, S. M., and Lebedeva, L., *Colloid J. (U. S. S. R.)*, **3**, 711(1937); through *Chem. Abstr.*, **32**, 6927(1938).
(3) Correll, J. T., U. S. pat. 2,507,244, 1950.

Solubility of Iodine in Glycol-Water Solutions*

By ARTHUR OSOL and CHARLES C. PINES

The solubility of iodine at 25° in ethylene glycol, propylene glycol, diethylene glycol, triethylene glycol, and dipropylene glycol increases in the order of solvents named, reaching a concentration of 88.16 Gm. of iodine in 100 cc. of dipropylene glycol solution. Addition of water to the respective glycols markedly reduces the solubility of iodine. Absorption spectra of iodine in the glycols, as well as in glycerin, indicate the presence of solvate complexes analogous to triiodide ion. The mechanism of solution of iodine in the solvents studied may involve a Lewis type of acid-base interaction, the iodine functioning as the acid and the solvent as the base.

MANY STUDIES of the solubility of iodine in various solvents have been made, the results of which have been summarized by Seidell (1) and in part by Hildebrand and Scott (2). The latter have used certain of these data in developing theoretical solubility generalizations. Iodine is of particular interest because some of its solutions are violet, while others are brown; the former are commonly considered to represent unsolvated systems while the latter are of the solvated type.

While data on the solubility of iodine in propylene glycol have been reported by Gershenfeld and Witlin (3), no study appears to have been made of the solubility in a series of glycols. This paper reports such a study, and also presents some evidence bearing on the nature of glycol solutions of iodine.

EXPERIMENTAL AND RESULTS

Materials.—Resublimed iodine, Baker and Adamson U. S. P. grade, was used without further purification when it was established that the solubility of a resublimed portion of that material in distilled water was exactly the same as the solubility of the original iodine.

The several glycols (ethylene, diethylene, triethylene, propylene, dipropylene) were the regular commercial grades produced by the Carbon and Carbide Chemicals Corp. and generously donated by the producer. Analyses by the manufacturer indicated the limits of foreign glycols to be as follows: in ethylene glycol, traces of diethylene glycol; in diethylene glycol, 0.5% of ethylene glycol and 0.5% or less of triethylene glycol; in triethylene glycol, no ethylene glycol, 0.5% of diethylene glycol, and 1.0% or less of tetraethylene glycol; in propylene glycol, traces of dipropylene glycol; in dipropylene glycol, 1.0% or less of propylene glycol and no tripropylene glycol. The content of water in these glycols, as determined in our laboratory by Karl Fischer titration, was as follows: ethylene, 0.24%; diethylene, 0.30%; triethylene, 0.21%; propylene, 0.25%; dipropylene, 0.19%.

The glycerin was of U. S. P grade (obtained from Harshaw Chemical Co.), and contained 4.95% of water, as determined by Karl Fischer titration.

Solubility Determination.—With the exception of solutions of iodine in distilled water, which were equilibrated both from undersaturated and supersaturated states, all other saturated solutions were prepared by rotating bottles containing solvent and an excess of powdered iodine for forty-eight hours in a water bath maintained at 25° ± 0.01°. The glass stoppers of the bottles were sealed into position with paraffin.

The water-glycol and water-glycerin solutions used as solvents were prepared by measuring into a dry volumetric flask the requisite volume of water, at 25°, then diluting to the mark with the glycol or glycerin; final adjustment of volume was made after the flask and contents had been kept at 25° for thirty minutes.

After the solutions were saturated, portions were removed by pipetting, particles of iodine being excluded by connecting to the tip of the pipette a constricted piece of glass tubing containing a pledget of cotton. At least two portions were taken for analysis and one for determination of specific gravity. The portions for analysis were placed in weighed flasks containing potassium iodide, weighed, diluted with water, and titrated with sodium thiosulfate volumetric solution. For low concentrations of iodine, 0.005 *N* sodium thiosulfate was used; for higher concentrations of iodine, 0.1 *N* solution was used. The size of the sample was adjusted so as to require at least 10 cc. of volumetric solution. Starch was used as indicator.

Solubility in Water.—In order to have complete data for the water-glycol systems, the solubility of iodine in distilled water was also determined. Solutions were prepared with hot water and with water at 25°, both being allowed to come to equilibrium during forty-eight hours or longer. Soda-lime glass containers and Pyrex glass bottles were used in preparing both solutions. Solutions were prepared with iodine U. S. P. as supplied and also from a resublimed sample of the same iodine. In one series of experiments the residue of iodine remaining after a saturated solution had been prepared was equilibrated with a fresh portion of water, and the residue from this second solution was employed in preparing a third. The results of all determinations agreed within 0.5%, the average corresponding to a solubility of 0.03354 Gm. of iodine per 100 cc. of solution, or of 2,980 cc. of water being required to dissolve 1 Gm. of iodine (the U. S. P. states that 2,950 cc. of water is needed to dissolve 1 Gm. of iodine).

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Solubility in Water-Glycol Systems.—Saturation of the pure glycols was considered to have been attained when successive additions of powdered iodine, followed by forty-eight-hour periods of rotation, yielded constant solubility (within 0.5% of the solubility observed with the next smaller amount of iodine). At least two separate solutions of each glycol were prepared; the measurements of solubility agreed to within 0.5% in these solutions. Usually only one solution was prepared for the determination of the solubility in water-glycol solutions, since the maximum amount of iodine needed to saturate the solutions was known.

The results of the solubility determinations in the water-glycol solutions, at 25°, are given in Tables I to V, while those in water-glycerin solutions are given in Table VI (see also Fig. 1).

TABLE I.—SOLUBILITY OF IODINE IN WATER-ETHYLENE GLYCOL SOLUTIONS

Concn. of Glycol, % (v/v)	Density Satd. Soln. at 25°	Iodine, Gm./100 Cc.
15	1.0194	0.0518
30	1.0388	0.0893
45	1.0564	0.1584
60	1.0793	0.4277
75	1.0972	1.043
90	1.1269	3.394
100	1.1605	7.163

TABLE II.—SOLUBILITY OF IODINE IN WATER-DIETHYLENE GLYCOL SOLUTIONS

Concn. of Glycol, % (v/v)	Density Satd. Soln. at 25°	Iodine, Gm./100 Cc.
15	1.0198	0.0609
30	1.0427	0.1189
45	1.0676	0.3234
60	1.0922	1.067
75	1.1283	3.986
90	1.2078	13.72
100	1.2742	17.49

Spectrophotometric Data.—Because of the wide range of solubility of iodine in the several glycols and in glycerin, it was decided to make a qualitative spectrophotometric survey of dilutions of these solutions to determine if there were any differences in the position of absorption peaks. The saturated solutions were diluted with the respective glycols or with glycerin to give convenient optical density readings. A Beckman spectrophotometer was used, measurements being made in the region of 320–600 mμ.

All solutions, including that in glycerin, exhibited maximum absorption only at approximately 358–360 mμ in the range studied. No difference in the position of the absorption peak was observed in solutions freshly prepared and those which were several weeks old. It is noteworthy that the absorption peak shown by a solution of iodine in water at 470 mμ was altogether absent in the solutions in the glycols and in glycerin. The peaks seen at still longer wave lengths in solvents yielding violet solutions with iodine were likewise absent. All our solutions were brown.

DISCUSSION

The high solubility of iodine in pure glycol solutions may be the result of the simultaneous occurrence of two or more different interactions. It appears likely that a solvation type interaction occurs, in which a reversible complex between iodine and solvent is formed. Such solvation interactions have occupied the attention of many investigators, but the nature of these interactions is still not known with certainty.

What appears to be a plausible explanation of at least some of these solvation interactions has been proposed by Benesi and Hildebrand (4). They suggest that iodine-solvent complexes may result from an acid-base interaction involving sharing of electron pairs, according to the G. N. Lewis defini-

TABLE III.—SOLUBILITY OF IODINE IN WATER-TRIETHYLENE GLYCOL SOLUTIONS

Concn. of Glycol, % (v/v)	Density Satd. Soln. at 25°	Iodine, Gm./100 Cc.
30	1.0523	0.1773
45	1.0737	0.4806
60	1.1031	1.704
75	1.1547	6.740
90	1.2759	21.93
95	1.3301	29.26
100	1.3854	36.71

TABLE IV.—SOLUBILITY OF IODINE IN WATER-PROPYLENE GLYCOL SOLUTIONS

Concn. of Glycol, % (v/v)	Density Satd. Soln. at 25°	Iodine, Gm./100 Cc.
45	1.0336	0.2124
60	1.0422	0.6174
75	1.0537	1.874
90	1.0815	5.907
95	1.1029	8.835
100	1.1345	13.01

tion. The Benesi-Hildebrand hypothesis states that iodine functions as an acid by serving as an electron-pair acceptor (presumably through preliminary formation of I^+I^-), while the solvent functions as a base by serving as an electron-pair donor. Since the oxygen atoms of the solvent glycols function as the electron-pair donors, increasing their number should enhance interaction with iodine. But this is not the sole factor to be considered, as replacement of hydrogen atoms by methyl or other hydrocarbon groups probably enhances the electron-donating ability of the solvent. On the basis of these considerations it would appear that the electron-donating ability of the glycols should increase in this order: ethylene, propylene, diethylene, triethylene, dipropylene. The extent of the solvation-type interaction with iodine might be expected to increase in the same order. Actually the solubility of iodine in these solvents does increase in this order.

The observation that the absorption spectra of solutions of iodine in the pure glycols and in glycerin show a characteristic absorption maximum at 358–360 mμ suggests that the nature of the iodine-

solvent interaction is in all cases of the same type, even though there is an approximately 80-fold difference in the solubility of iodine in glycerin and in dipropylene glycol. To some extent at least, this interaction appears to be involved when iodine is dissolved in alcohol, since such solutions show an absorption peak in the same region, although there is another peak at approximately 445 mμ which is not observed in either the glycol or the glycerin solutions.

TABLE V.—SOLUBILITY OF IODINE IN WATER-DIPROPYLENE GLYCOL SOLUTIONS

Concn. of Glycol, % (v/v)	Density Satd. Soln. at 25°	Iodine, Gm./100 Cc.
45	1.0364	0.6368
60	1.0570	2.738
75	1.1221	10.78
90	1.4500	48.78
95	1.6416	69.41
100	1.7804	88.16

In this connection, it is particularly noteworthy that the absorption spectrum of iodine in aqueous potassium iodide solution shows a peak at 353 mμ (5), which Benesi and Hildebrand attribute to triiodide ion formed by interaction of iodine, functioning as an acid, with iodide ion, functioning as a base. Stobbe and Schmidt (6) observed an absorption peak at 357 mμ in several solutions of iodine, which they attribute to formation of periodides such as hydrogen triiodide or other "addition compounds."

TABLE VI.—SOLUBILITY OF IODINE IN WATER-GLYCERIN SOLUTIONS

Concn. of Glycerin, % (v/v)	Density Satd. Soln. at 25°	Iodine, Gm./100 Cc.
30	1.0783	0.0780
60	1.1563	0.1890
75	1.1931	0.3351
90	1.2283	0.6633
95	1.2398	0.8524
100	1.2510	1.103

Since our glycol and glycerin solutions show intense absorption peaks at practically the same wave length, it is likely that formation of a triiodide or "periodide" occurs, but whether this is a solvation reaction of the Lewis acid-base type, or an instance of an irreversible chemical reaction or even some other type of reaction, is not clearly apparent. For example, the absorption spectrum of iodine in acetone is also characterized by a single, intense absorption peak at 363 mμ, which Benesi and Hildebrand (7) now attribute to triiodide ion resulting from an initial instantaneous reaction of acetone with iodine to form iodoacetone and iodide ion, which latter then establishes equilibrium with iodine to form I_3^-. They cite as evidence in favor of this explanation the fact that while the purified acetone used in preparing their solutions was neutral to litmus, a solution of iodine in acetone gave a distinct acid reaction due to formation of hydrogen and iodide ions.

Since our solutions gave no acid reaction during the time required to saturate them with iodine, it appears unlikely that a "chemical" reaction is responsible for the high solubility of iodine (solutions which had aged for several months did show a slight acid reaction). On the other hand, we observed that the glycols behave as bases toward crystal violet indicator, since the latter gradually changes to its acid color when the glycol is titrated with a solution of perchloric acid in dioxane. This would support the hypothesis that an acid-base type solvation interaction may be primarily responsible for the solubility of iodine in glycols and in glycerin. Since a by-product of such an interaction is very likely to be iodide ion (from the I^+I^- initially formed from a molecule of iodine), subsequent reaction with molecular iodine would form triiodide ion, which would account for the similarity of the absorption spectrum of iodine in glycols with that of iodine in an aqueous iodide solution.

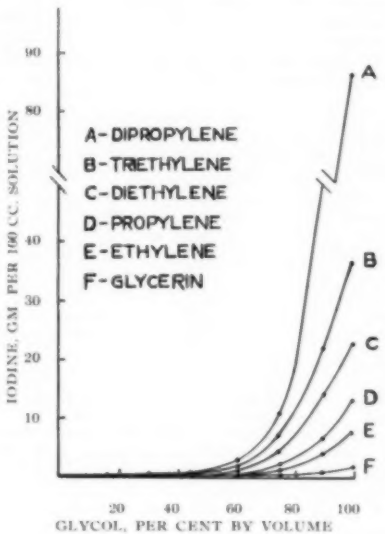

Fig. 1.—Solubility of iodine in various glycol solutions.

SUMMARY

1. The solubility of iodine in water-glycol and in water-glycerin solutions has been determined.

2. The qualitative spectrophotometric absorption characteristics of these solutions in the wave length region of 320–600 mμ have been determined.

3. Evidence is cited to support the hypothesis that a Lewis acid-base type solvation interaction may be primarily responsible for the solubility of iodine in glycols and in glycerin.

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Potential Ergot Substitutes: Esters and Amides of *beta*-Amino Acids*

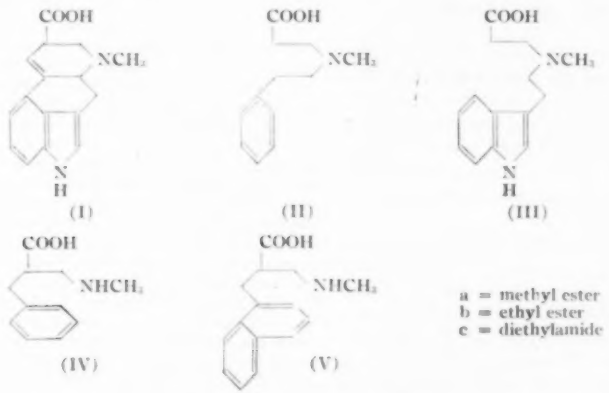
By P. E. NORRIS and F. F. BLICKE

Six esters and amides of derivatives of β-alanine which are related to lysergic acid have been prepared and tested for oxytocic activity. None of these products possess a significant oxytocic activity. A method for the preparation of N-methyltryptamine is described.

ERGOT has been used in medicine for many years as an oxytocic drug. Of the six pharmacologically active alkaloids which are found in ergot, the most important clinically is ergonovine. The high cost, the uncertainties of an agricultural source, and the low yields of the alkaloids are disadvantages which make it desirable to discover a synthetic substitute for ergot or, particularly, for ergonovine. All of the ergot alkaloids are substituted amides of lysergic acid (I);[1] ergonovine, the 2-(1-hydroxy)-propylamide, is the simplest of these alkaloids.

The purpose of this investigation was to synthesize amides and also esters of compounds (II–V) which represent fragments of the lysergic acid molecule in the hope that some of these products might possess oxytocic activity.

Various modified fragments of the lysergic acid molecule have been synthesized previously; it was claimed that some of the compounds are active oxytocics (1–7). Baltzly, Dvorkovitz, and Phillips (3) found, during the course of an extensive investigation of derivatives of phenylethyl-β-alanine, that in laboratory animals, the esters of several of the compounds prepared possess oxytocic activity equal to that of the ethanolamide of the same compound.



* Received August 22, 1952, from the College of Pharmacy, University of Michigan, Ann Arbor.
Presented to the Scientific Section, A. PH. A., Philadelphia meeting, August, 1952.

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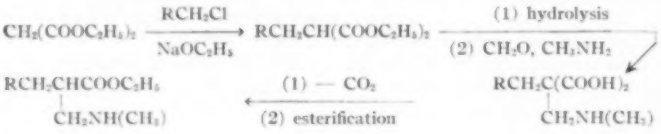
The methyl ester and the diethylamide of compound II were prepared by the condensation of N-methylphenylethylamine with methyl acrylate and N,N-diethylacrylamide, respectively, and were isolated as hydrobromides. The methyl ester and the diethylamide of III were obtained in the same manner except that N-methyltryptamine was substituted for N-methylphenylethylamine. In the latter case the diethylamide was purified by chromatography with an alumina column; a stable oxalate salt was obtained for use in pharmacological tests. The required N-methyltryptamine was obtained by the following procedure: 3-indoleacetonitrile→ tryptamine → N-formyltryptamine → N-methyltryptamine.

Ethyl esters of compounds IV and V were prepared by the alkylation of malonic ester with either benzyl chloride or α-chloromethylnaphthalene, followed by hydrolysis of the esters, isolation of the substituted malonic acids, Mannich reactions of the acids with formaldehyde and methylamine, decarboxylation, and then re-esterification of the remaining carboxyl group.



R = phenyl or α-naphthyl

The yields from this method are very poor and the products are unusually difficult to purify, due to the fact that the products from the Mannich reactions tend to dissociate spontaneously into a substituted acrylic acid, carbon dioxide, and methylamine. In fact, this process has been used as a preparative method to obtain α-substituted acrylic acids (8).

Pharmacologic data indicated that none of the esters or amides of compounds II–V which were prepared possess a significant oxytocic action when compared to the clinically used oxytocics. However, the diethylamide of N-methyl-N-[β′-(3-indolyl)-ethyl]-β-alanine (IIIc) appeared to have an oxytocic activity approximately ten times stronger than that of the diethylamide of N-methyl-N-(β′-phenylethyl)-β-alanine (IIc).

EXPERIMENTAL

Methyl Ester of N-Methyl-N-(β′-phenylethyl)-β-alanine Hydrobromide (IIa).—This compound was prepared by the general method of Baltzly, *et al.* (3). The hydrobromide was recrystallized from absolute alcohol-ether and melted at 145–147°; yield, 4.5 Gm. (50%).

Anal.—Calcd. for $C_{13}H_{20}O_2NBr$: N, 4.63; Br, 26.44. Found: N, 4.49; Br, 26.01.

N,N-Diethylacrylamide.—To freshly distilled acrylyl chloride[2] (20.4 Gm.) in 150 cc. of benzene, cooled in an ice-bath, there was added dropwise 23.8 Gm. of diethylamine. Diethylamine hydrochloride precipitated. After two hours at room temperature, the mixture was filtered and the filtrate was fractionated; yield, 20 Gm. (70%); b. p. 82–88° (9 mm.); reported (11) b. p. 95° (19 mm.); n_D^{25} 1.4670; reported (11) n_D^{25} 1.4672.

Diethylamide of N-Methyl-N-(β′-phenylethyl)-β-alanine Hydrobromide (IIc).—A solution of 3.5 Gm. of N-methylphenylethylamine and 7 Gm. of N,N-diethylacrylamide in 100 cc. of benzene was refluxed for fifteen hours. An ethereal solution of hydrogen bromide was added dropwise to the cooled solution until the mixture remained cloudy. Upon refrigeration, an oily layer separated, and after a day or two, crystals formed on the walls of the flask. The oily layer was seeded with a few crystals and refrigeration was continued. The solid after recrystallization from benzene melted at 69–73°; yield, 5 Gm. (80%).

Anal.—Calcd. for $C_{15}H_{25}ON_2Br$: N, 8.16; Br, 23.27. Found: N, 7.86; Br, 23.04.

Tryptamine.—A solution of 7.6 Gm. (0.2 mole) of lithium aluminum hydride in 300 cc. of dry ether was prepared in a three-necked flask equipped with a stirrer, reflux condenser, and dropping funnel. To the stirred solution was added dropwise a solution of 15.6 Gm. (0.1 mole) of indoleacetonitrile[3] in 120 cc. of dry ether. The addition required about one and one-half hours. The mixture was refluxed for one-half hour and then cooled. Fifty cubic centimeters of water was added dropwise, followed by 75 cc. of 10% sodium hydroxide solution. The ether layer was separated and the aqueous gel was extracted with ether. The combined ether extracts were dried over magnesium sulfate and the ether was removed immediately by distillation under reduced pressure. The oily residue was distilled and the fraction which boiled at 155–160° (0.5 mm.) or at 130–135° (0.05 mm.) was retained. The distillate solidified and was recrystallized from benzene; m. p. 113–115°; reported (12) m. p. 114–115°. The hydrochloride, recrystallized from alcohol-ether melted at 245–247°; reported (12) m. p. 248–249°. The picrate melted at 242–244°; reported (12) m. p. 242–243°.

N-Formyltryptamine.—A mixture of 6.0 Gm. (0.037 mole) of tryptamine and 1.9 Gm. (0.042 mole) of formamide was heated in an oil bath (125–130°) for four hours and stirred frequently.

[2] Acrylyl chloride was obtained from acrylic acid by the method of Rehberg, Dixon, and Fisher (9). Acrylic acid was prepared from α-chloropropionic acid by the procedure of Moureu, Murat, and Tampier (10).

[3] Prepared from indole by the method of Majima and Hoshino (12).

The product boiled at 195–205° (0.02 mm.); reported (13) b. p. 190–200° (0.04 mm.). The yield of viscous oil was 6.2 Gm. (86%).[4]

N-Methyltryptamine.—N-Formyltryptamine, 13.3 Gm., was reduced with 5.5 Gm. of lithium aluminum hydride by the procedure described above. The oily product crystallized from an ethereal solution which was cooled in a dry ice-acetone bath. The N-methyltryptamine melted at 85–87°; reported (14) m. p. 89–90°; yield, 10.5 Gm. (86%). The picrate, recrystallized from methanol-ethanol, melted at 186–188°; reported (14) m. p. 190–191°. The hydrochloride, recrystallized from alcohol-ether, melted at 175–176°; reported (15) m. p. 180°. The hydrobromide, recrystallized from alcohol-ether, melted at 156–158°.

Methyl Ester of N-Methyl-N-[β′-(3-indolyl)-ethyl]-β-alanine Hydrochloride (IIIa).—A mixture of 1.74 Gm. of N-methyltryptamine, 1.74 Gm. of methyl acrylate, and 30 cc. of benzene was allowed to remain at room temperature for twelve hours and then was refluxed for one hour. The benzene solution was cooled and an ethereal solution of hydrogen bromide was added dropwise, until no more precipitate formed. The liquid was decanted from the gummy precipitate and the latter was recrystallized from alcohol-ether. The product melted at 110–112°; yield, 2 Gm. (60%).

Anal.—Calcd. for $C_{14}H_{21}O_2N_2Br$: C, 52.78; H, 6.20; N, 8.21; Br, 23.4. Found: C, 52.81; H, 6.23; N, 8.33; Br, 23.6.

Diethylamide of N-Methyl-N-[β′-(3-indolyl)-ethyl]-β-alanine Oxalate (IIIc).—A solution of 1.74 Gm. of N-methyltryptamine, 2.54 Gm. of N,N-diethylacrylamide, and 6 drops of Triton "B" in 60 cc. of benzene was refluxed for eight hours. The solution was cooled and then was poured over a column of alumina (¾ in. x 23 in.) which had been previously moistened with benzene. The column was eluted with a 2% methanol-in-benzene solution. Ten-cubic centimeter portions were collected in tared beakers and then were evaporated to dryness. A graphic comparison of the cubic centimeters collected with the milligrams of residue produced a curve with two peaks. The first peak was found to represent the excess of starting material and the second peak to represent the product. Four of the residues, which were the bulk of the material represented by the second peak, were combined. An attempt to "distill evaporatively" the viscous syrup (0.5 to 1.0 mm.) by the use of a sublimation apparatus was unsuccessful. The hydrobromide could not be induced to crystallize. The syrup was dissolved in ether and treated with a 2% ethereal solution of oxalic acid. The acid oxalate was crystallized from absolute alcohol-ether and melted at 133–136°; yield, 1.5 Gm. (50%).

Anal.—Calcd. for $C_{20}H_{29}O_5N_3$: C, 61.36; H, 7.46; N, 10.73. Found: C, 61.22; H, 7.61; N, 10.48.

α-Benzyl-α-methylaminomethylmalonic Acid.—This compound was prepared by the general method of Mannich and Ganz (8). Both formaldehyde (37%) and methylamine (40%) were used in the form of aqueous solutions. As soon as the exothermic reaction subsided the solid product precipitated. After several hours at room temperature the mixture was filtered; if a longer time elapsed the precipitated material became oily. The crude product melted at 125–130° (dec.); reported (8) m. p. 150° (dec.) for recrystallized material.

Ethyl Ester of N-Methyl-α-benzyl-β-alanine Hydrochloride (IVb).—The preceding compound was placed in a centrifuge tube and heated in an oil bath which was maintained at temperatures between 125 and 130°. The solid melted and was stirred occasionally with a glass rod. The evolved gases were basic. After fifteen minutes the viscous oil was cooled and dissolved in alcoholic hydrogen chloride. The solution was allowed to remain at room temperature for fifteen hours and was then concentrated to a syrup in a stream of air. The syrup was diluted with an equal volume of absolute alcohol, and two volumes of ether were added. Methylamine hydrochloride crystallized from the solution and was removed by centrifugation. The decanted solution was diluted to several times its volume with ether and was then refrigerated. An oily layer formed and crystals separated in the alcohol-ether layer; the mixture was decanted from the oil. The crystals were then separated from the liquid by centrifugation, and after they had been dried *in vacuo*, melted at 76–85°. A second crop of crystals was obtained in the same manner when the alcohol-ether solution was diluted with more ether and refrigerated; m. p. 73–82°. The combined crystalline material was recrystallized from absolute alcohol-ether; m. p. 109–111°.

Anal.—Calcd. for $C_{13}H_{20}O_2NCl$: C, 60.57; H, 7.82; N, 5.43; Cl, 13.75. Found: C, 60.62; H, 7.66; N, 5.69; Cl, 13.74.

Ethyl Ester of N-Methyl-α-(α′-naphthylmethyl)-β-alanine Hydrochloride (Vb).—Diethyl α-naphthylmethylmalonate (16) was hydrolyzed to the corresponding acid. The latter substance was then subjected to the above series of reactions. The product, recrystallized from alcohol-ether, melted at 168–170°.

Anal.—Calcd. for $C_{17}H_{22}O_2NCl$: C, 66.33; H, 7.20; N, 4.55. Found: C, 66.11; H, 7.34; N, 4.79.

[4] Schöpf and Steuer (13) reported that the distillate slowly solidified and melted at 76°.

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The Identification and Determination of N-Allylnormorphine Hydrochloride*

By W. J. SEAGERS, J. D. NEUSS, and W. J. MADER†

N-Allylnormorphine hydrochloride, a potent antagonist for the respiratory depressive effect of morphine, can be identified by infrared absorption spectrophotometry in the form of the crystalline material or an injectable solution. Polarography and ultraviolet absorption spectrophotometry provide alternate reliable assay methods for both forms of N-allylnormorphine hydrochloride.

N-ALLYLNORMORPHINE HYDROCHLORIDE (1), an alkaloid recently synthesized from morphine and a potent antagonist for the respiratory depressive effect of the parent compound, morphine (2, 3), has recently been marketed under the trade name Nalline[1] hydrochloride as an injectable solution. Methods of identification and assay were necessary for the chemical control of the crystalline substance before subdivision and of the marketed product after subdivision and sterilization.

It was found that the infrared spectrum offered a reliable means of identification of N-allylnormorphine hydrochloride as well as the detection of the presence, if any, of morphine or normorphine hydrochlorides both in the crystalline substance and in the injectable solution.

Ultraviolet spectrophotometry and polarography provide reliable alternate means of assay for N-allylnormorphine hydrochloride in the crystalline material and in the injectable solution.

INFRARED SPECTROPHOTOMETRY

The infrared spectra of N-allylnormorphine hydrochloride and morphine hydrochloride are sufficiently different, due to the substitution of an allyl group in N-allylnormorphine for the N-methyl group of morphine, so that the detection of morphine in the presence of N-allylnormorphine is possible. In Fig. 1, the infrared spectra of both compounds over the range 8–12 μ are shown. It can be easily seen that N-allylnormorphine hydrochloride exhibits maxima at 9.70, 10.04, 10.20, 10.42, 10.56, 10.90, 11.06, and 11.37 μ which are not exhibited by morphine hydrochloride, and exhibits no maxima at 9.80, 10.32, 10.70, and 11.48 μ where morphine hydrochloride does exhibit maxima. Both compounds produce maxima at 7.59 and 7.90 μ, not shown in Fig. 1.

The operations necessary for the isolation of N-allylnormorphine hydrochloride from the parenteral solution, which contains sodium bisulfite and sodium citrate in addition to the alkaloid, change the infrared spectrum significantly. Thus, in Fig. 2, the infrared spectrum of N-allylnormorphine hydrochloride after isolation is compared with the spectra of morphine hydrochloride and normorphine hydrochloride which have been carried through the same operations. The significant features are the presence of a maximum in the N-allylnormorphine hydrochloride spectrum at 11.60 μ which is absent in the spectra of morphine hydrochloride and normorphine hydrochloride, and the absence of a maximum at 11.47 μ which is present in the spectra of morphine hydrochloride and normorphine hydrochloride.

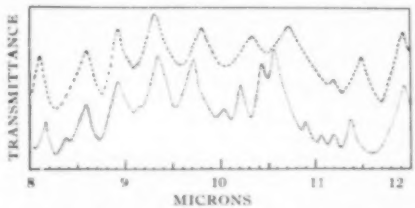

Fig. 1.—Infrared Spectra, - - - - Morphine·HCl, ... *N*-Allylnormorphine·HCl. About 20 mg. of each alkaloid (untreated) mulled with 0.05 ml. liquid petrolatum.

Procedure.—The samples of the pure alkaloids were prepared for infrared examination by grinding about 20 mg. of the alkaloid hydrochloride in a mortar and pestle and mulling with about 0.05 ml. of liquid petrolatum. All infrared spectra were recorded on a single beam model IR-2 Beckman spectrophotometer, using a sodium chloride prism and a sodium chloride cell. The wave-length scale was calibrated using an atmospheric carbon dioxide band at 4.22 μ and a benzene maxima at 9.64 μ.

The N-allylnormorphine hydrochloride in parenteral solution was isolated by evaporating to dryness 4–6 ml. of the parenteral solution containing 5 mg. of N-allylnormorphine hydrochloride per ml., selectively extracting the alkaloid hydrochloride with absolute methanol, evaporating the methanol filtrate to dryness, and drying the residue at 100° for ten minutes. The dried residue was mulled with liquid petrolatum, and the infrared spectrum was recorded as described above. Morphine hydrochloride and normorphine hydrochloride were carried through

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† The authors gratefully acknowledge the aid of Mr. E. Schultz who performed the infrared measurements and Mr. A. Doering who performed the ultraviolet measurements.
[1] Registered trade mark of Merck and Co., Inc., Rahway, N. J.

the same operations from dissolving in absolute methanol and their spectra recorded as described above.

ULTRAVIOLET SPECTROPHOTOMETRY

The ultraviolet absorption spectrum of N-allylnormorphine hydrochloride in water solution exhibits a maximum at 285 mμ and a minimum at about 260 mμ, as shown in Fig. 3 (solid line). Addition of acid causes practically no change in the spectrum, but alkali causes a considerable increase in absorption and shifts the maximum to 298 mμ and the minimum to about 278 mμ.

As might be predicted from the chemical structure, the spectrum is not specific for N-allylnormorphine. Morphine, normorphine, and related compounds give similar spectra, since they are essentially identical with respect to their U. V. chromophore, the hexahydrophenanthrene ring. Therefore, identification and differentiation by this means are not possible. However, samples which have been adequately identified by infrared spectrophotometry may be assayed by the ultraviolet absorption method.

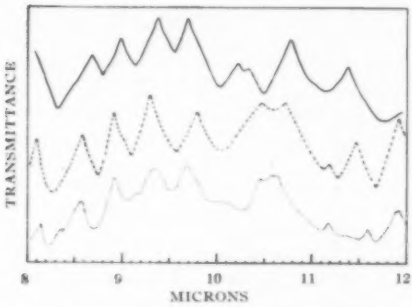

Fig. 2.—Infrared Spectra, —— Normorphine-HCl, - - - - Morphine-HCl, ... N-Allylnormorphine-HCl. Ca. 20 mg. of each alkaloid (obtained by evaporating methanol solutions of each alkaloid and drying at 100°) mulled with about 0.05 ml. of liquid petrolatum.

Samples of crystalline N-allylnormorphine hydrochloride were assayed by the following procedure. An accurately weighed sample of about 25 mg. was diluted with water to 200 ml. in a volumetric flask. The absorbancy was determined at 1 mμ intervals in the vicinity of 285 mμ with a Beckman DU spectrophotometer, using 1-cm. quartz cells and water as reference liquid. An absorption maximum should be found at 285 ± 1 mμ. The assay was calculated as follows:

$$\text{Per cent N-allylnormorphine hydrochloride} = \frac{4{,}545\ A_{285}}{S}$$

where A_{285} is the absorbancy at 285 mμ and S is the sample weight in milligrams. The factor, 4,545, is based on the dilution and on the extinction coefficient for the pure substance, $A^{1\%,\ 1\ cm.}_{285} = 44.0$.

Sterilized parenteral solutions could not be accurately assayed by the above method. It was found that sterilization by autoclaving produced a slight but measurable absorbancy in the region 310–360 mμ (Fig. 3, broken line). This was evidently due to slight decomposition, since unautoclaved solutions show no absorbancy in this region. The absorbancy at 285 mμ was also enhanced by autoclaving, so that high results were obtained if the assay was calculated by the above method. The error was of the order of 6% relative. By taking the absorbancy at 310 mμ as a measure of the enhancement produced by autoclaving, the assay was corrected empirically by subtracting the absorbancy at 310 mμ from the absorbancy at 285 mμ. Details of the assay are as follows:

Procedure.—A sample of 1.00 ml. of the parenteral solution was diluted to 25.0 ml. with water. The absorbancies were determined at 310-mμ and at 1-mμ intervals in the vicinity of 285 mμ with a Beckman DU spectrophotometer, using 1-cm. quartz cells and water as reference liquid. An absorption maximum should be found at 285 ± 1 mμ. The assay was calculated as follows:

$$\text{Mg. of N-allylnormorphine hydrochloride per ml.} = 11.4\ (A_{285} - A_{310})$$

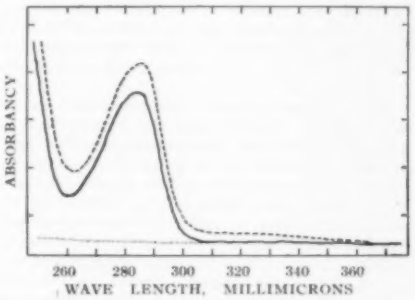

Fig. 3.—Ultraviolet spectra of N-allylnormorphine hydrochloride in water solution; dashed line, parenteral solution; full line, pure substance; dotted line, water blank.

POLAROGRAPHY

N-Allylnormorphine hydrochloride exhibits a polarographic wave in 2 *M* lithium chloride which has a half-wave potential of −1.6 v. *vs.* S. C. E. This wave is suitable for assay purposes since the height of the wave is directly proportional to the concentration. The wave shape, Fig. 4, indicative of an irreversible process, and the *p*H independence of the half-wave potential lead to the conclusion that the electrode reaction is the reduction of the double bond of the allyl group. The fact that neither morphine hydrochloride nor normorphine hydrochloride exhibits a polarographic wave under these conditions supports this conclusion. The polarographic wave is diffusion-controlled since its height is directly proportional to the square root of the mercury column height, i. e., $id/h^{1/2}$ is a constant within ±2% (4).

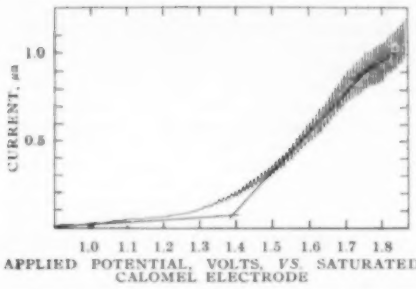

Fig. 4.—Typical polarographic curve for N-allylnormorphine hydrochloride in 2 *M* lithium chloride.

Procedure.—Preparation of the sample solutions was as follows: for the crystalline substance, about 125 mg. of sample was accurately weighed into a 25-ml. volumetric flask and diluted to volume with water. Ten milliliters of this solution was diluted to 250 ml. with water in a volumetric flask and the resulting solution was used as the sample solution. For the injectable solution, which contained 5 mg. of N-allylnormorphine hydrochloride per ml. and was packaged in 2-ml. ampuls, 1.00 ml. of the solution was pipetted into a 25-ml. volumetric flask, diluted to volume with water, and used as the sample solution.

As a standard solution in the analysis of crystalline material, N-allylnormorphine hydrochloride of known purity (99.8%), from phase solubility determinations (5), was treated in the same manner as the crystalline substance. This standard solution was prepared fresh daily. As a standard for the parenteral solution, a synthetic solution was prepared identical in composition with the parenteral solution, i. e., each ml. of standard solution contained 5.0 mg. of N-allylnormorphine hydrochloride of known purity (99.8%), 2.0 mg. of sodium bisulfite, and 15 mg. of sodium citrate, and was treated in the same manner as the parenteral solution. This standard solution was also prepared fresh daily.

In each case the solution added to the polarographic cell was prepared from the sample solutions in the following manner: 5.0 ml. of the sample solution was mixed with 15.0 ml. of 2.65 *M* lithium chloride solution and shaken well. About 10 ml. of this solution was placed in an H-type polarographic cell (6), which was totally enclosed in a glass envelope maintained at 25° by circulating water, and then was purged of oxygen by passing nitrogen, purified by passage through alkaline pyrogallol, through the solution for five minutes. A conventional dropping mercury electrode was used in conjunction with a Sargent Model XX polarograph. Polarograms were recorded over the potential range −0.5 to −2.0 v. *vs.* S. C. E. at a suitable sensitivity. Wave heights were determined and calculated in microamperes. The appropriate values were substituted in the following formulas. In order for the formulas to be valid, the height of the mercury column above the tip of the capillary must remain constant for the sample and standard solutions. For the pure substance:

$$\%\ \text{N-allylnormorphine hydrochloride} = \frac{\text{height sample wave } (\mu a.)}{\text{height std. wave } (\mu a.)} \times \%\ \text{purity std.} \times \frac{\text{mg. std.}}{\text{mg. sample}}$$

For the injectable solution:

$$\text{Mg. N-allylnormorphine hydrochloride per ml.} = \frac{\text{height sample wave } (\mu a.)}{\text{height std. wave } (\mu a.)} \times \frac{\%\ \text{purity std.}}{100} \times \frac{\text{mg. std.}}{25}$$

The precision of these assays is ±3%; hence the moisture contents of the pure substance and standard were neglected since they have averaged about 0.1% as determined by the Karl Fischer method.

RESULTS AND DISCUSSION

A comparison of the results obtained with the ultraviolet assay method and with the polarographic method on production lots of the autoclaved injectable solution is shown in Table I.

Table I.—Comparison of U. V. and Polarographic Assay of Production Lots of Autoclaved Injectable Solutions of Nalline Hydrochloride

Lot No.	U. V. Assay, Mg./Ml.	Polarographic Assay, Mg./Ml.
1	5.0	4.9
2	5.1	4.8
3	5.1	5.0
4	5.1	4.9
5	5.1	5.0
6	5.3	4.9
7	5.4	5.0
8	5.2	4.9
9	5.1	5.0
10	5.1	4.8

It can be seen from Table I that the ultraviolet and polarographic assay results agree within their respective precisions. Since the two methods are essentially independent, one depending on the adsorption of radiant energy by the large hexahydrophenanthrene ring and the other on the reduction of the small allyl group, the validity of the assays is established. Since the polarographic assay for the injectable solution can be standardized with material of known purity, the ultraviolet assay can be standardized by comparison.

Thus, the ultraviolet method because of its speed and more readily available equipment may be the assay method of choice.

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Isoquinoline Derivatives As Local Anesthetics*

By ELVIN L. ANDERSON, JAMES W. WILSON, and GLENN E. ULLYOT

The preparation, properties, and local anesthetic activity of a series of 1-aminoalkoxyisoquinoline derivatives and the requisite 1-chloroisoquinoline intermediates are reported. In general, local anesthetic activity, as measured on the rabbit cornea, is maximal when the isoquinoline is substituted in the 3-position by a butyl group and in the 1-position by a 3-(1-methylpiperidyloxy) group.

In a previous paper (1) we described a number of aminoalkoxyisoquinoline compounds which possessed pronounced local anesthetic activity. This series of compounds has been extended in an effort to determine the structural requirements for maximum activity. Variations in the nature of the 1-aminoalkoxy group and in the size of the 3-substituent have been studied, and a few compounds with substituents in other positions of the isoquinoline nucleus have been evaluated.

For the most part the isoquinoline intermediates were prepared by methods already described. In several instances, however, we have employed a new method of synthesis involving the ring closure of phenethyl isocyanates with aluminum chloride.

The intermediate chloroisoquinolines are summarized in Table I. Tables II, III, and IV record some of the physical properties of the free bases of those compounds which were tested for local anesthetic activity. The test solutions were prepared by dissolving the free base in one equivalent of dilute hydrochloric acid, adding one equivalent of a sodium acetate buffer, and diluting to the desired concentration (*p*H 3.5 to 6.5).

The duration of anesthesia recorded in Tables II, III, and IV was determined[1] on the rabbit cornea in the usual way (2) by noting the period of time required for the return of the winking reflex after the application of a given concentration of the agent being tested. The figures given represent average times for a total of 6 to 10 animals. We have compared the duration of anesthesia obtained with a 0.1 per cent solution when these data were available, but we have made no attempt to report the detailed pharmacological data for these compounds. This will be done at a later date.

* Received August 27, 1952, from the Research and Development Division, Smith, Kline and French Laboratories, Philadelphia, Pa.

[1] We are indebted to Dr. Edwin J. Fellows, Mr. Edward Macko, and Mr. Keith F. Killam of the Smith, Kline and French Laboratories for permission to reproduce these data.

EXPERIMENTAL

Preparation of Amino Alcohols.—When not available commercially, these intermediates were prepared by methods described in the literature.[2]

N-(β-Hydroxyethyl)-2,2,4,6-tetramethylpiperidine.—A mixture of 70.5 Gm. (0.5 mole) of 2,2,4,6-tetramethylpiperidine and 20.1 Gm. (0.25 mole) of ethylene chlorohydrin was maintained at the reflux temperature for eleven hours; it was then cooled and treated with 200 cc. of 40% sodium hydroxide. The resulting oil was extracted with ether, dried, and freed of solvent under reduced pressure. Distillation of the residue yielded 16.5 Gm. of recovered 2,2,4,6-tetramethylpiperidine and 33.9 Gm. (37%) of product as a colorless oil; b.p. 126–128° (11 mm.), n_D^{25} 1.4762.

Anal.—Calcd. for $C_{11}H_{23}NO$: C, 71.29; H, 12.51. Found: C, 71.19; H, 12.43.

N-(β-Hydroxyethyl)-2,6-dimethylpiperidine.—Using 68.0 Gm. (0.6 mole) of 2,6-dimethylpiperidine and 24.2 Gm. (0.3 mole) of ethylene chlorohydrin, the above procedure was repeated to give 28.0 Gm. (60%) of product; b. p. 81–82° (2 mm.), n_D^{25} 1.4817.

Anal.—Calcd. for $C_9H_{19}NO$: C, 69.74; H, 12.18. Found: C, 69.74; H, 11.84.

N-(β-Hydroxyethyl)-2-hexylpiperidine.—The above procedure was repeated using 85 Gm. (0.5 mole) of 2-hexylpiperidine and 20.2 Gm. (0.25 mole) of ethylene chlorohydrin to give 31.8 Gm. (60%) of product; b. p. 100–104° (0.3 mm.), n_D^{25} 1.4760.

Anal.—Calcd. for $C_{13}H_{27}NO$: C, 73.18; H, 12.76. Found: C, 73.22; H, 12.49.

Preparation of Substituted Isoquinolones.—Most of these intermediates were prepared by the arrangement of the appropriate 1-amino-1-(3-phthalidyl)-alkanes (3). We have found that substituted isoquinolones may also be readily prepared by treating phenethyl isocyanates with aluminum chloride to effect ring closure to give tetrahydroisoquinolones which, in turn, are readily dehydrogenated to give the desired compounds.[3]

Ethyl N-[β-(3,4-Dimethoxyphenyl)-ethyl] Carbamate (4).—Over a period of forty-five minutes, a solution of 54.5 Gm. (0.5 mole) of ethyl chlorocarbonate in 100 cc. of dry benzene was added at 50°, with stirring, to a solution of 181 Gm. (1.0 mole) of 3,4-dimethoxyphenethylamine in 250 cc. of dry benzene. After stirring at 50° for thirty minutes, the mixture was extracted with water and saturated sodium bicarbonate solution. The benzene was removed by distillation and the product distilled; yield, 100 Gm. (79%); b. p. 176–178° (4 mm.).

Anal.—Calcd. for $C_{13}H_{19}NO_4$: C, 61.64; H, 7.56. Found: C, 61.32; H, 7.53.

6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolone-1 (4, 5).—A solution of 51 Gm. (0.2 mole) of ethyl

[2] We wish to thank Dr. Kenneth N. Campbell of the University of Notre Dame for generous samples of 1,2,6-trimethyl-4-piperidinol, 1-ethyl-2,6-dimethyl-4-piperidinol, and 1-propyl-2,6-dimethyl-4-piperidinol.

[3] We wish to thank Dr. T. E. Aschner of our laboratories for suggesting and developing experimental conditions for this reaction.

N-[β-(3,4-dimethoxyphenyl)-ethyl]carbamate in a mixture of 100 Gm. (0.7 mole) of phosphorus oxychloride and 7 Gm. of phosphorus pentoxide was heated at gentle reflux for three hours. The solution was hydrolyzed with ice and made alkaline with excess 40% sodium hydroxide. The product was extracted with chloroform. From this solution 35 Gm. (85%) of product, m. p. 138–145°, was obtained. After two crystallizations from methyl ethyl ketone the product melted at 155 to 156.5°.

Anal.—Calcd. for $C_{11}H_{13}NO_3$: C, 63.75; H, 6.32. Found: C, 63.75; H, 6.07.

Subsequent runs yielded a product melting at 172° which is in agreement with the value reported by Späth and Dowbrowsky (4).

6,7-Dimethoxy-1,2-dihydroisoquinolone-1.—A mixture of 2.0 Gm. (0.0096 mole) of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolone-1 and 0.3 Gm. of a 30% palladium-on-charcoal catalyst was placed in a bath at 200°. A slow stream of dry nitrogen was then bubbled into the mixture and the temperature was increased to 240° over a period of thirty minutes. The product was crystallized from chloroform; yield, 1.4 Gm. (70%); m.p. 213–217°. Further recrystallization raised the melting point to 237–238°.

Anal.—Calcd. for $C_{11}H_{11}NO_3$: C, 64.38; H, 5.40. Found: C, 64.41; H, 5.44.

3-Benzyl-1,2,3,4-tetrahydroisoquinolone-1.—1,3-Diphenylisopropylamine (6) was prepared in 55% yield from dibenzyl ketone by means of the Leuckart reaction. The amine was dissolved in nitrobenzene and saturated with hydrogen chloride; phosgene was then passed through the refluxing solution for two hours. The residue remaining after removal of solvent was distilled under reduced pressure to give a 57% yield of 1,3-diphenylisopropyl isocyanate, b. p. 197° (15 mm.). The isocyanate was characterized by treating with ammonia to give the unsymmetrical urea; m. p. 138 to 138.5°.

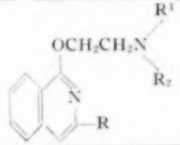
Anal.—Calcd. for $C_{16}H_{18}N_2O$: N, 11.02. Found: N, 11.13.

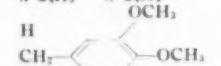
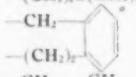
TABLE I.—1-CHLORO-x-SUBSTITUTED ISOQUINOLINES

Substituent (s)	Formula	Prep. Method	M. P., °C.	B. P., °C.	Pressure, Mm.	n_D^{25}	t_f, °C.	Yield, %	Analysis, % Calcd C	Calcd H	Found C	Found H
3-Isobutyl	$C_{13}H_{14}ClN$[a]	A	.....	125–126	0.3	1.5841	23	63				
3-*n*-Amyl	$C_{14}H_{16}ClN$	A	.....	142–144	2	1.5874	24	78	71.94	6.90	71.93	6.76
3-*n*-Hexyl	$C_{15}H_{18}ClN$	A	.....	151–153	0.3	1.5701	23	62	72.73	7.32	72.67	7.52
3-Phenyl	$C_{15}H_{10}ClN$	A	76–78[b]	.....	.....	.....	.....	81				
4-Phenyl	$C_{15}H_{10}ClN$	A	114–115.5	.....	.....	.....	.....	100	75.16[c]	4.21	74.75	4.35
3-Benzyl	$C_{16}H_{12}ClN$	A	.....	180–182	0.5	1.6442	23	74	75.74	4.77	75.57	4.85
4-Bromo	C_9H_5BrClN	B	97.5–98[d]	.....	.....	.....	.....	85	44.57	2.08	44.69	1.99
5-Nitro	$C_9H_5ClN_2O_2$	[e]	183–184	.....	.....	.....	.....	57				
5-Amino	$C_9H_7ClN_2$	[f]	175–176	.....	.....	.....	.....	77	60.51	3.95	60.54	4.19
3-Ethyl-5-nitro	$C_{11}H_9ClN_2O_2$	[e]	92.5–93.5	.....	.....	.....	.....	89	55.82	3.83	55.72	3.86
3-Ethyl-5-amino	$C_{11}H_{11}ClN_2$	[f]	124–125	.....	.....	.....	.....	83	63.92	5.37	64.08	5.45
7-*n*-Butyl	$C_{13}H_{14}ClN$	A	.....	134–136	0.5	1.5872	23	85	71.06	6.42	71.01	6.61
6,7-Dimethoxy	$C_{11}H_{10}ClNO_2$	A	155–160	.....	.....	.....	.....	[g]				
3-Methyl-7-methoxy	$C_{11}H_{10}ClNO$	A	111–112	.....	.....	.....	.....	39	63.62	4.85	63.62	4.79
3-Ethyl-6,7-dimethyl	$C_{13}H_{14}ClN$	A	.....	155–156	2.5	1.6050	25	33	[h]			
3-Methyl-4-*n*-propyl	$C_{13}H_{14}ClN$	B	.....	143–145	1.5	1.6005	20	78	71.06	6.42	71.10	6.84
3-Methyl-4-*n*-butyl	$C_{14}H_{16}ClN$	B	.....	158–160	2	1.5874	28	51	71.95	6.90	71.99	6.65
3-Ethyl-7-amino	$C_{11}H_{11}ClN_2$	A	145–145.5	.....	.....	.....	.....	73	63.92	5.37	64.10	5.58

[a] Lehmkuhl, J. M., *Ber.*, **30**, 896(1897); reported a b. p. of 298–300°. [b] Gabriel, S., *ibid.*, **18**, 3470(1885); reported a m. p. of 77–78°. [c] Calcd.: N, 5.84. Found: N, 5.79. [d] Elpern, B., and Hamilton, C. S., *J. Am. Chem. Soc.*, **68**, 1436 (1946); reported a m. p. of 97°. [e] The appropriate 1-chloroisoquinoline was nitrated using the procedure described by Le Fevre, C. G., and Le Fevre, R. J. W., *J. Chem. Soc.*, **1935**, 1472. Ref[e] reported a m. p. of 183–184° for 1-chloro-5-nitroisoquinoline. [f] Reduction of the appropriate nitro compound was effected at room temperature over Raney nickel catalyst and platinic chloride. Ref[e] reported a m. p. of 154° for 1-chloro-5-aminoisoquinoline. [g] Characterized as the picrate salt, m. p. 190–191°. *Anal.*—Calcd. for $C_{17}H_{13}N_5O_9$: N, 12.37. Found: N, 12.16. [h] Characterized as the picrate salt, m. p. 114.5–115°. *Anal.*—Calcd. for $C_{18}H_{15}N_5O_9$: N, 12.48. Found: N, 12.38.

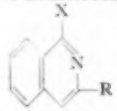
TABLE II.—AMINOETHOXYISOQUINOLINES



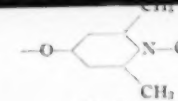
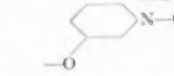
	R	R¹	R²	Formula	B. P., °C.	Pressure, Mm.	n_D^{25}	t, °C.	Yield, %	Analysis, % Calcd. C	Calcd. H	Found C	Found H	Duration of Anesthesia of 0.1% Solution, Min.
1.	CH_3	CH_3	CH_3	$C_{14}H_{18}N_2O$[a]	132–134	1	1.5637	20	87	73.01	7.88	72.90	7.95	24
2.	C_2H_5	H	H	$C_{13}H_{16}N_2O$	177–178[b]	0.5	1.6266	20	61	72.19	7.45	72.24	7.59	0
3.	C_2H_5	H	C_2H_5	$C_{15}H_{20}N_2O$[c]	158–164	4	....	....	81	73.73[d]	8.25	73.27	8.18	153
4.	C_2H_5	CH_3	CH_3	$C_{15}H_{20}N_2O$	153–156	2	1.5580	20	80	73.73[e]	8.25	73.69	8.04	183
5.	C_2H_5	CH_3	CH_2CH_2OH	$C_{16}H_{22}N_2O_2$	169–172	0.3	1.5668	23	59	70.04	8.08	69.99	8.17	104
6.	C_2H_5	n-C_3H_7	n-C_3H_7	$C_{19}H_{28}N_2O$	172–175	2	1.5375	20	58	75.95	9.39	75.71	9.06	125
7.	C_2H_5	n-C_4H_9	n-C_4H_9	$C_{21}H_{32}N_2O$	188–190	2	1.5297	20	58	76.78	9.82	76.78	9.88	111
8.	C_2H_5	H		$C_{22}H_{28}N_2O_3$	220–225	0.5	1.5945	25	50	72.10	7.15	71.95	7.11	137
9.	n-C_4H_9	CH_3	CH_3	$C_{17}H_{24}N_2O$	155–157	3	1.5486	20	87	74.95	8.88	74.99	8.81	514
10.	n-C_4H_9	H	$CH_2C_6H_5$	$C_{22}H_{26}N_2O$	202–204[f]	0.3	1.5839	23	57	79.00	7.84	78.85	7.65	174[g]
11.	n-C_4H_9	—$(CH_2)_5$—		$C_{20}H_{32}N_2O$[h]	189–191	1	1.5543	23	76	76.88[b]	9.03	76.59	9.03	480[i]
12.	n-C_4H_9	n-C_3H_7	n-C_3H_7	$C_{21}H_{32}N_2O$[j]	177–178	1	1.5293	25	78	76.78	9.82	76.88	9.77	159
13.	n-C_5H_{11}	CH_3	CH_3	$C_{18}H_{26}N_2O$[k]	162–164	1	1.5440	25	70	75.48	9.15	75.34	9.09	253[l]
14.	C_6H_5	CH_3	CH_3	$C_{19}H_{20}N_2O$[l]	198–200	1	1.6310	24	79	78.05	6.90	78.20	6.69	70
15.	n-C_6H_{13}	CH_3	CH_3	$C_{19}H_{28}N_2O$	175–176	0.4	1.5388	23	83	75.95	9.39	76.01	9.36	233[l]
16.	iso-C_4H_9	CH_3	CH_3	$C_{17}H_{24}N_2O$[m]	140–141	0.4	1.5451	23	81	74.96	8.88	74.83	8.87	61
17.	$CH_2C_6H_5$	CH_3	CH_3	$C_{20}H_{22}N_2O$[n]	188–189	0.4	1.5935	23	70	78.40	7.24	78.28	7.38	....
18.	C_2H_5	CH_3	CH_3	$C_{20}H_{28}N_2O$	176–177	0.4	1.5592	23	63	76.88	9.03	76.96	9.25	161
19.	C_2H_5	—$CH(CH_2)_5CH$— C_2H_5 —$CH(CH_2)_5$—		$C_{24}H_{36}N_2O$	201–203	0.4	1.5428	23	59	78.21	9.85	78.23	9.84	138
20.	C_2H_5	—$(CH_2)_2O(CH_2)_2$—		$C_{17}H_{22}N_2O_2$	170–172	0.3	1.5688	23	76	71.30	7.74	71.28	7.80	66
21.	C_2H_5			$C_{22}H_{24}N_2O$	207–208	0.3	1.6033	23	61	79.48	7.28	79.54	7.22	21
22.	C_2H_5	CH_3 CH_3 $(CH_2)_2$ —CH CH_2CHCH_2—		$C_{22}H_{32}N_2O$	186–189	0.4	1.5497	23	44	77.59	9.47	77.03	9.29	42

[a] Monopicrate, m. p., 179–180°. *Anal.*—Calcd. for $C_{20}H_{21}N_5O_8$: C, 52.28; H, 4.61. Found: C, 52.20; H, 4.68. [b] M. p., 82–84°. [c] Monohydrochloride, m. p., 116–126°. *Anal.*—Calcd. for $C_{15}H_{21}Cl_2N_2O$: Cl, 12.63; C, 64.16; H, 7.54. Found: Cl, 12.46; C, 64.14; H, 7.54. [d] Calcd.: N, 11.47. Found: N, 11.70. [e] Calcd.: N, 11.47. Found: N, 11.35. [f] 0.01% solution. [g] 0.05% solution. [h] Monopicrate, m. p. 110–111°. *Anal.*—Calcd. for $C_{26}H_{31}N_5O_8$: N, 12.93. Found: N, 13.00. [i] Calcd.: N, 8.97. Found: N, 8.92. [j] Monopicrate, m. p. 82–82.5°. *Anal.*—Calcd. for $C_{27}H_{34}N_4O_8$: N, 12.56. Found: N, 12.79. [k] Monopicrate, m. p., 107–108°. *Anal.*—Calcd. for $C_{24}H_{29}N_5O_8$: N, 13.59. Found: N, 13.55. [l] Monopicrate, m. p., 182–182.5°. *Anal.*—Calcd. for $C_{25}H_{25}N_5O_8$: N, 13.43. Found: N, 13.55. [m] Monopicrate, m. p., 132–133°. *Anal.*—Calcd. for $C_{23}H_{27}N_5O_8$: N, 13.97. Found: N, 14.05. [n] Monopicrate, m. p., 143–144°. *Anal.*—Calcd. for $C_{26}H_{28}N_4O_8$: N, 13.08. Found: N, 13.27. [o] Skita, A., *Ber.*, **57B**, 1977 (1924).

TABLE III.—1-SUBSTITUTED ISOQUINOLINES

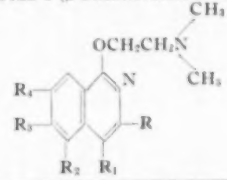


	R	X	Formula	B. P., °C.	Pressure, Mm.	n_D^t	t, °C.	Yield, %	Analysis, % Calcd. C	Calcd. H	Found C	Found H	Duration of Anesthesia of 0.1% Soln., Min.
23.	C_2H_5	$-OCHCH_2NHCH(CH_3)_2$ (CH₃)	$C_{17}H_{24}N_2O$[a]	130–134	2	1.5448	25	63	74.90[b]	8.88	74.50	8.41	155
24.	C_2H_5	$-OCH_2CHN(C_2H_5)_2$ (CH₃)	$C_{18}H_{28}N_2O$[c]	185–187	7	1.5382	25	84	75.48	9.15	75.42	9.22	58
25.	C_2H_5	$-OCH(CH_2)_3N(C_2H_5)_2$ (CH₃)	$C_{20}H_{30}N_2O$	177–179	0.5	1.5321	25	63	76.39	9.62	76.28	9.47	128
26.	C_2H_5	$-OCH_2CH_2CH_2N(C_2H_5)_2$	$C_{19}H_{28}N_2O$	166–169	4	....	..	52	75.48[d]	9.15	74.97	9.23	142
27.	C_2H_5	$-O-C_6H_4-N(CH_3)-CH_2-CH_2$	$C_{17}H_{22}N_2O$	184–185	4	....		70	75.52[e]	8.20	75.07	7.94	182
28.	C_2H_5	$O-CH(CH_2-CH_2)_2NCH_2$	$C_{19}H_{24}N_2O$[f]	177–178	1	1.5778	25	79	76.99	8.16	77.06	8.01	108[g]
29.	C_2H_5	$-S-CH_2CH_2N(C_2H_5)_2$[h]	$C_{17}H_{24}N_2S$[i]	179–182	1	1.5870	20	66	....	..	...	..	61
30.	n-C_4H_9	$-O-C_6H_4-N(CH_3)-CH_2-CH_2$	$C_{19}H_{28}N_2O$[j]	168–170	1	1.5546	25	84	76.47	8.78	76.44	8.88	480
31.	n-C_4H_9	$-O-C_6H_3(CH_3)-N(CH_3)-CH_2-CH_2$	$C_{21}H_{30}N_2O$	174–176	1	1.5492	23	46	77.24	9.26	77.29	9.11	480[k]
32.	n-C_4H_9	$O-CH(CH_2-CH_2)_2NCH_2$ (CH₃)	$C_{21}H_{32}N_2O$	189–190	0.4	1.5658	23	70	77.73	8.70	77.67	8.60	360
33.	n-C_4H_9	$NHCH_2CH_2N(C_2H_5)_2$	$C_{19}H_{29}N_3$	168–170	0.3	1.5626	23	39	76.20	9.76	76.12	9.51	195
34.	C_2H_5	$-O(CH_2)_3N(C_2H_5)_2$	$C_{19}H_{28}N_2O$	154–156	0.3	1.5400	23	33	75.95	9.39	76.05	9.41	68
35.	n-C_4H_9	$-O-C_6H_4-N(C_3H_7)-CH_2-CH_2$	$C_{21}H_{30}N_2O$[l]	199–201	1.0	1.5456	24	36	77.24	9.26	77.59	9.01	....
36.	n-C_4H_9	$-OCH(CH_2)_3N(C_2H_5)_2$ (CH₃)	$C_{22}H_{34}N_2O$	170–172	1.0	1.5255	23	63	77.14	10.01	77.09	9.88	....
37.	n-C_4H_9	$-O-C_6H_4-N(CH_2C_6H_5)-CH_2-CH_2$	$C_{25}H_{30}N_2O$[m]	237–240	0.5	1.5801	24	40	80.17	8.07	79.93	7.57	....
38.	n-C_4H_9	$-O-C_6H_3(CH_3)-N(C_2H_5)-CH_2-CH_2$	$C_{22}H_{32}N_2O$	188–190	1.0	1.5482	23	70	77.60	9.47	77.71	9.48	360[g]

39.	*n*-C_4H_9		$C_{25}H_{34}N_2O$	199–201	1.0	1.5408	24	43	77.92	9.67	77.89	9.73	.....
40.	*n*-C_4H_9		$C_{19}H_{26}N_2O^a$	170–171	0.4	1.5561	24	45	76.47	8.78	76.33	8.66	308[g]

[a] Monopicrate, m. p. 126–126.5°. *Anal.*—Calcd. for $C_{31}H_{37}N_9O_8$: C, 55.08; H, 5.43. Found: C, 54.94; H, 5.13. [b] Calcd: N, 10.29. Found: N, 10.47. [c] Monopicrate, m. p. 108–109°. *Anal.*—Calcd. for $C_{32}H_{39}N_9O_8$: C, 55.91; H, 5.67. Found: C, 56.03; H, 5.59. [d] Calcd: N, 9.78. Found: N, 9.84. [e] Calcd: N, 10.36. Found: N, 10.69. [f] Monopicrate, m. p. 185–186°. *Anal.*—Calcd. for $C_{32}H_{37}N_9O_8$: N, 13.33. Found: N, 13.27. [g] 0.01% solution. [h] Albertson, N. F., and Clinton, R. O., *J. Am. Chem. Soc.*, **67**, 1222(1945). [i] Calcd: S, 11.12. Found: S, 11.09. [j] Monopicrate, m. p. 116–117°. *Anal.*—Calcd. for $C_{29}H_{29}N_9O_8$: N, 13.28. Found: N, 13.29. [k] Test terminated at this time. At a concentration of 0.01%, the duration of anesthesia was 124 minutes. [l] Monopicrate, m. p. 165–166°. *Anal.*—Calcd. for $C_{21}H_{29}N_9O_8$: N, 12.61. Found: N, 12.85. [m] Monopicrate, m. p. 140–141°. *Anal.*—Calcd. for $C_{21}H_{31}N_9O_8$: N, 11.60. Found: N, 11.75. Monohydrochloride, m. p. 203–204°. *Anal.*—Calcd. for $C_{19}H_{31}N_3OCl$: Cl, 8.63. Found: Cl, 8.43. Succinate, m. p. 135–136°. *Anal.*—Calcd. for $C_{31}H_{43}N_3O_9$: N, 5.69. Found: N, 5.82. [n] R. Paul, *Compt. rend.*, **221**, 412(1945). [o] Monopicrate, m. p. 123–124°. *Anal.*—Calcd. for $C_{31}H_{39}N_9O_8$: N, 13.28. Found: N, 13.30.

TABLE IV.—X-SUBSTITUTED-1-(β-DIMETHYLAMINOETHOXY) ISOQUINOLINES



	R	R[1]	R[2]	R[3]	R[4]	Formula	B. P., °C.	Pressure, Mm.	n_D^t	t, °C.	Yield, %	Analysis, % Caled. C	Caled. H	Found C	Found H	Duration of Anesthesia of 0.1% Soln., Min.
41.	H	Br	H	H	H	$C_{15}H_{15}BrN_2O^a$	146–148	1	1.5942	25	56	52.89[b]	5.12	53.35	4.99	15
42.	CH_3	*n*-C_3H_7	H	H	H	$C_{17}H_{24}N_2O$	167–169	1	1.5663	23	88	74.96	8.88	74.87	8.93	0
43.	CH_3	*n*-C_4H_9	H	H	H	$C_{18}H_{26}N_2O$	156–160	1	1.5520	20	83	75.48	9.15	75.29	8.88	80
44.	C_2H_5	H	H	CH_3	CH_3	$C_{17}H_{24}N_2O$	174–175	3	1.5584	25	67	74.96	8.88	74.82	8.62	24
45.	H	H	H	OCH_3	OCH_3	$C_{16}H_{20}N_2O_2^c$	181–182	1	1.5788	23	62	65.19	7.30	65.41	7.09	16
46.	C_2H_5	H	NH_2	H	H	$C_{19}H_{21}N_3O^d$	187–188	0.5	1.5989	23	61	69.46	8.16	69.28	8.35	151
47.	H	H	NH_2	H	H	$C_{26}H_{17}N_3O^e$	169–171[f]	0.3	1.6189	23	50	67.50	7.41	67.58	7.42	0
48.	H	H	H	H	*n*-C_4H_9	$C_{17}H_{24}N_2O^g$	145–147	0.3	1.5505	23	82	74.96	8.88	74.94	8.62	0
49.	H	C_6H_5	H	H	H	$C_{19}H_{20}N_2O$	228–229	5	1.6119	24	72	78.05	6.90	77.96	6.93	...
50.	C_2H_5	H	$NHCOCH_3$	H	H	$C_{17}H_{22}N_2O_2$	..... [h]	..	.....	..	100	67.75[i]	7.69	67.52	7.65	0
51.	CH_3	H	H	H	OCH_3	$C_{18}H_{20}N_2O_2^j$	159–161	1	1.5651	23	75	69.20	7.74	69.21	7.79	31[k]

[a] Monopicrate, m. p. 201–202°. *Anal.*—Calcd. for $C_{21}H_{23}BrN_8O_8$: N, 13.36. Found: N, 13.26. [b] Calcd: N, 9.49. Found: N, 9.63. [c] Monopicrate, m. p. 200–201°. *Anal.*—Calcd. for $C_{31}H_{33}N_9O_9$: N, 13.86. Found: N, 13.87. [d] Monopicrate, m. p. 150–151°. *Anal.*—Calcd. for $C_{31}H_{35}N_9O_8$: N, 17.21. Found: N, 17.14. [e] Monopicrate, m. p. 198–199°. *Anal.*—Calcd. for $C_{31}H_{33}N_9O_8$: N, 18.26. Found: N, 18.06. [f] M.p. 48–50°. [g] Monopicrate, m. p. 89–90°. *Anal.*—Calcd. for $C_{23}H_{27}N_9O_8$: N, 13.97. Found: N, 14.13. [h] M.p. 114–115°. Obtained by acetylation of compound 46. [i] Calcd: N, 13.94. Found: N, 13.70. [j] Monopicrate, m. p. 170–171°. *Anal.*—Calcd. for $C_{31}H_{35}N_9O_9$: N, 14.31. Found: N, 14.44. [k] 0.01% solution.

To 28 Gm. (0.08 mole) of aluminum chloride dissolved in 150 cc. of nitrobenzene was added 20.0 Gm. (0.08 mole) of 1,3-diphenylisopropyl isocyanate, and the resulting mixture was stirred at 75° for three hours; it was then cooled and slowly poured into 250 cc. of ice water. Chloroform (250 cc.) was added and the organic layer was separated and dried. The solvents were removed by distillation under reduced pressure to give an oil which was triturated with petroleum ether to give 10 Gm. (50%) of product; m. p. 144–146°. A portion was recrystallized from dilute alcohol; m. p. 148 to 148.5°.

Anal.–Calcd. for $C_{16}H_{13}NO$: C, 80.98; H, 6.37. Found: C, 81.09; H, 6.35.

3-Benzyl-1,2-dihydroisoquinolone-1.—A mixture of 6.3 Gm. (0.026 mole) of 3-benzyl-1,2,3,4-tetrahydroisoquinolone-1 and 1.5 Gm. of 10% palladium-on-charcoal catalyst was heated at 305–320° for six hours while a stream of dry nitrogen was passed through the molten mass. The mixture was cooled and treated with 225 cc. of boiling alcohol, and the catalyst was separated by filtration. Chilling the filtrate yielded 3.6 Gm. of product, m. p. 191.5 to 193°, and concentration to 50 cc. gave an additional 0.8 Gm., m. p. 191 to 192.5°, for a total yield of 4.4 Gm. (72%). A portion was recrystallized from 99% isopropanol; m. p. 192–193°.

Anal.—Calcd. for $C_{16}H_{13}NO$: C, 61.68; H, 5.57. Found: C, 61.69; H, 5.44.

Addition of water to the filtrate yielded 1.2 Gm. of solid, m. p. 180–184°, which was primarily the product contaminated with a trace of tetrahydro material.

***p*-*n*-Butylbenzyl Cyanide.**—*n*-Butylbenzene was converted to *p*-*n*-butylbenzyl chloride according to the procedure of Kosolapoff (7). A mixture of 60 Gm. (0.33 mole) of *p*-butylbenzyl chloride, 45.5 Gm. (0.7 mole) of potassium cyanide, 910 cc. of alcohol, and 140 cc. of water was heated at the reflux temperature for twelve hours. The precipitated potassium chloride was separated by filtration and washed with several small portions of alcohol. The filtrate was concentrated to 200–250 cc. and then diluted with 500 cc. of cold water. The resulting oil was extracted into ether and dried. The solvent was removed by distillation under reduced pressure, and the residual oil was distilled *in vacuo* to give 43.2 Gm. (76%) of product as a colorless oil; b. p. 163–165° (19 mm.), n_D^{25} 1.5061.

A small portion of the above nitrile was refluxed for twenty minutes with 50% sulfuric acid. Upon cooling, a solid separated and was dissolved in dilute sodium hydroxide. The alkaline solution was washed with small portions of ether and then acidified to give *p*-*n*-butylphenylacetic acid, which was recrystallized from dilute alcohol; m. p. 73 to 74.5°.

β-(*p*-*n*-Butylphenyl)-ethylamine.—A solution of 112 Gm. (0.5 mole) of *p*-butylbenzyl cyanide in 725 cc. of methanol saturated with ammonia was reduced over Raney nickel catalyst at room temperature and an initial pressure of 1420 p. s. i. to give 106.8 Gm. (92%) of β-(*p*-*n*-butylphenyl)-ethylamine as a colorless oil; b.p. 146–151° (20 mm.), n_D^{25} 1.5105 to 1.5132. A portion was converted to the hydrochloride salt; m. p. 184 to 185.5°.

Anal.—Calcd. for $C_{12}H_{20}NCl$: Cl^-, 16.59. Found: Cl^-, 16.29.

β-(*p*-*n*-Butylphenyl)-ethyl Isocyanate.—To a mixture of 250 Gm. (2.5 mole) of phosgene and 500 cc. of sodium-dried toluene, contained in a 2-L. three-necked flask cooled in an ice bath and equipped with a dropping funnel, a mechanical stirrer, and a dry-ice condenser, was added 80 Gm. (0.45 mole) of β-(*p*-*n*-butylphenyl)-ethylamine mixed with 100 cc. of dry toluene over a period of one hour. The ice bath was removed and the mixture was stirred with careful warming to 40° to permit phosgene to escape When all of the excess phosgene had been removed, the reaction mixture was stirred at 80–85° for two hours. The clear solution was freed of solvent by distillation under reduced pressure and the residue was distilled at 16 mm. to give 82 Gm. (92%) of product as a colorless oil; b. p. 150–156°; n_D^{24} 1.5060 to 1.5068.

7-*n*-Butyl-1,2,3,4-tetrahydroisoquinolone-1.—To 33.7 Gm. (0.22 mole) of aluminum chloride dissolved in 125 cc. of nitrobenzene at 60° was added dropwise over a period of one hour a mixture of 19.8 Gm. (0.1 mole) of β-(*p*-butylphenyl)-ethyl isocyanate and 25 cc. of nitrobenzene. The addition was at such a rate that the temperature did not exceed 65° (the yield is greatly decreased at higher temperatures). The reaction mixture was stirred at 63° for one hour and was then cooled to 20° and slowly poured into 100 cc. of ice water. The organic layer was separated, diluted with 100 cc. of chloroform, washed with several 25-cc. portions of cold water, and dried. The solvents were removed *in vacuo* and the residue was distilled to give 18.0 Gm. (89%) of product as a viscous yellow oil; b. p. 180–183° (0.5 mm.), n_D^{24} 1.5545. After prolonged standing the product solidified; m. p. 45–46°.

Anal.—Calcd. for $C_{13}H_{17}NO$: C, 76.81; H, 8.43. Found: C, 76.65; H, 8.38.

7-*n*-Butyl-1,2-dihydroisoquinolone-1.—A mixture of 10.0 Gm. (0.049 mole) of 7-*n*-butyl-1,2,3,4-tetrahydroisoquinolone-1 and 1.5 Gm. of 10% palladium-on-charcoal catalyst was heated at 310–320° for four hours with a slow stream of nitrogen bubbling through the molten mass. After cooling, 100 cc. of boiling alcohol was added, and the catalyst was removed by filtration. The filtrate was diluted with water and cooled to give an oily yellow solid which was washed thoroughly with petroleum ether to give 5.5 Gm. (55%) of pale yellow crystals; m. p. 126–128°. Concentration of the filtrates gave 3.0 Gm. of recovered starting material as a viscous yellow oil. A sample of the solid was recrystallized from dilute alcohol and submitted for analysis; m. p. 127.5 to 129°.

Anal.—Calcd. for $C_{13}H_{15}NO$: C, 77.58; H, 7.51. Found: C, 77.74; H, 7.31.

***p*-Methoxyphenylisopropyl Isocyanate.**—To 320 Gm. (3.2 mole) of phosgene dissolved in 750 cc. of chlorobenzene was added a solution of 82.5 Gm. (0.5 mole) of *p*-methoxyphenylisopropylamine in 75 cc. of chlorobenzene over a period of two hours. Phosgene was then passed through the mixture for thirty minutes with stirring. The solution was warmed very slowly to permit excess phosgene to escape and then was heated at the reflux temperature for two hours. Removal of solid by filtration gave 23.2 Gm. of recovered *p*-methoxyphenylisopropylamine as the hydrochloride salt; m. p. 207–209°. The filtrate was freed of solvent by distillation under reduced pressure, and the residue was distilled to give 57.5 Gm. (60%) of the isocyanate as a colorless oil; b. p. 112–113° (3 mm.), 155–

150° (22–23 mm.); n_D^{23} 1.5168. Addition of a few drops of this material to concentrated ammonium hydroxide gave a white solid which recrystallized from dilute alcohol as white needles, m. p. 169–170°, having a strong anise-like odor.

3 - Methyl - 7 - methoxy - 1,2,3,4 - tetrahydroisoquinolone-1.—To 60 Gm. (0.45 mole) of aluminum chloride in 150 cc. of nitrobenzene at 43° was slowly added (two hours) 28.5 Gm. (0.15 mole) of *p*-methoxyphenylisopropyl isocyanate so that the temperature did not exceed 45° [the temperature appears to be quite critical since little or no yield of product is obtained at higher (65 to 80°) or lower (−15 to 20°) temperatures]. The reaction mixture was stirred at 42–45° for an additional two hours and was then cooled and slowly poured into 200 cc. of ice water. The separation of the organic layer was facilitated by the addition of 100 cc. of chloroform. The chloroform solution was washed well with cold water and dried, and the solvent was removed by distillation under reduced pressure to leave a gummy brown solid which was triturated with petroleum ether and dissolved in boiling 99% isopropyl alcohol. Cooling gave 16.3 Gm. (57%) of light brown solid; m. p. 138–141°. Recrystallization from 99% isopropyl alcohol (Darco) gave colorless prisms, m. p. 149–150°.

Anal.—Calcd. for $C_{11}H_{13}NO_2$: C, 69.09; H, 6.85. Found: C, 68.96; H, 6.59.

3-Methyl-7-methoxy-1,2-dihydroisoquinolone-1.—A mixture of 7.5 Gm. (0.039 mole) of 3-methyl-7-methoxy-1,2,3,4-tetrahydroisoquinolone-1 and 1.0 Gm. of 10% palladium-on-carbon catalyst was heated under a stream of nitrogen at 250–260° for four hours. The cooled mixture was dissolved in 75 cc. of boiling alcohol and the catalyst was removed by filtration. Cooling the filtrate gave 3.1 Gm. of material, m. p. 139 to 140.5°; concentration and further cooling yielded an additional 3.7 Gm., m. p. 139–141°, for a total of 6.8 Gm. (90%). A portion was recrystallized from alcohol, m. p. 140–141°.

Anal.—Calcd. for $C_{11}H_{11}NO_2$: C, 69.82; H, 5.86. Found: C, 69.62; H, 5.63.

Preparation of 1-Chloroisoquinolines.—The physical properties and analytical data for these compounds are given in Table I.

Method A.—These compounds were prepared as previously described (1) by the action of phosphorus oxychloride upon the appropriately substituted isoquinolone.

Method B.—The appropriate isoquinoline N-oxide or N-oxide hydrochloride, prepared essentially by the method described by Robinson (8), was heated at reflux temperature with excess phosphorus oxychloride for five to seven hours. The solution was hydrolyzed with ice, made basic with 40% sodium hydroxide, and extracted with ether. The ether was removed and the residue was purified by distillation under reduced pressure or by crystallization.

The position of the entering chlorine atom was established by the following series of reactions. A sample of 1-chloro-3-ethylisoquinoline (1) was dehalogenated over Raney nickel catalyst to give an 87% yield of 3-ethylisoquinoline; picrate, m. p. 173 to 173.5°; reported, 171–172° (9). When 3-ethylisoquinoline was treated with hydrogen peroxide and glacial acetic acid in the usual way (8), a 68% yield of 3-ethylisoquinoline N-oxide hydrochloride, m. p. 203 to 203.5°, was obtained.

Anal.—Calcd. for $C_{11}H_{12}NOCl$: C, 63.01; H, 5.77; Cl^-, 16.91. Found: C, 62.79; H, 5.71; Cl^-, 16.75.

The 3-ethylisoquinoline N-oxide, when treated with excess phosphorus oxychloride in the manner described above, gave a 61% yield of 1-chloro-3-ethylisoquinoline. This compound, when refluxed with dilute sulfuric acid, gave a quantitative yield of 3-ethyl-1,2-dihydroisoquinolone-1, m. p. 139–140°, undepressed upon admixture with an authentic sample of 3-ethyl-1,2-dihydroisoquinolone-1 (3).

3-Methyl-4-butylisoquinoline N-Oxide Hydrochloride.—A solution of 125 cc. of glacial acetic acid, 50 Gm. (0.5 mole) of 30% hydrogen peroxide, and 50 Gm. (0.25 mole) of 3-methyl-4-*n*-butylisoquinoline[4] was allowed to stand at 60° for five days. At the end of this time, most of the acetic acid was removed by distillation under reduced pressure. The residual oil was diluted with a mixture of 100 cc. of methanol and 200 cc. of ether and then was treated with excess ethereal hydrogen chloride to give 52 Gm. (84%) of white powder; m. p. 196–198°.

Anal.—Calcd. for $C_{14}H_{18}ClNO$: C, 66.79; H, 7.21; Cl^-, 14.08. Found: C, 66.88; H, 7.18; Cl^-, 13.76.

3 - Methyl - 4 - *n* - propylisoquinoline N - Oxide.—Treating 139 Gm. (0.75 mole) of 3-methyl-4-*n*-propylisoquinoline[4] essentially as described above gave 42.4 Gm. (28%) of pale yellow powder, m. p. 114–115°.

Anal.—Calcd. for $C_{13}H_{15}NO$: C, 77.58; H, 7.51; N, 6.96. Found: C, 77.56; H, 7.53; N, 7.14.

3-Methyl-4-*n*-propylisoquinoline N-Oxide Hydrochloride.—3-Methyl-*n*-propylisoquinoline N-oxide (20.1 Gm.; 0.1 mole) was dissolved in 75 cc. of methanol and treated with excess ethereal hydrogen chloride to give 23.1 Gm. (97%) of white powder; m. p. 212–215°.

Anal.—Calcd. for $C_{13}H_{16}ClNO$: C, 65.67; H, 6.78; Cl, 14.92. Found: C, 65.46; H, 6.67; Cl, 15.01.

Preparation of Aminoalkyl Ethers.—These compounds were all prepared as previously described (1). Their properties are presented in Tables II, III, and IV.

Cleavage of Aminoalkyl Ethers.[5]—Dilute (2–4%) aqueous solutions of the aminoalkyl ethers, containing one or two equivalents of hydrochloric acid (*p*H = 1.5 to 3.0), upon long standing (at room temperature) slowly deposit crystals of the substituted 1,2-dihydroisoquinolone-1. At higher temperatures the rate of cleavage is much more rapid, and nearly quantitative yields of the isoquinolone are obtained. Solutions buffered to a *p*H of 3.5 to 6.2 with sodium acetate are quite stable for long periods of time at room temperature or higher. Under these circumstances the ethers can be recovered from the solution in yields of 90–100%, as the picrate salts.

(*a*) A solution of 1.00 Gm. (0.00409 mole) of 1-(β-dimethylaminoethoxy)-3-ethylisoquinoline (compound 4) in 20.4 cc. (0.00818 mole) of 0.400 *N* hydrochloric acid was diluted to 50 cc. (*p*H = 1.5) and heated at 73° for four hours. The resulting mixture was cooled to 10° and the 1,2-dihydro-3-ethyl-

[4] This material was provided by Reilly Tar and Chemical Company. The structure has not been definitely established but is believed to be correct.

[5] For a similar cleavage in the quinoline series, see Bogert and May, *J. Am. Chem. Soc.*, **31**, 507 (1909).

isoquinolone-1 (2) was collected, yielding 0.60 Gm. (84%), m. p. 137–140°.

(*b*) A solution of 1.00 Gm. (0.00409 mole) of 1-(β-dimethylaminoethoxy)-3-ethylisoquinoline (compound 4) in 10.2 cc. of 0.40 *N* hydrochloric acid containing 0.5566 Gm. (0.00409 mole) of sodium acetate was diluted to 50 cc. (*p*H = 6.0) and heated at 73° for ten hours. The addition of 20 cc. (0.00460 mole) of a solution of picric acid in alcohol (0.053 Gm./cc.) yielded 1.8 Gm. (92%) of the picrate derivative; m. p. 151–153°. Recrystallization from isopropanol gave 1.6 Gm.; m. p., 153–154°.

Anal.—Calcd. for $C_{21}H_{23}N_5O_8$: C, 53.27; H, 4.90; N, 14.79. Found: C, 53.15; H, 4.98; N, 14.62.

DISCUSSION

The topical anesthetic activity of these compounds was greatly affected by structural variations in the molecule. The nature of the substituent in the 3-position seemed to be of major importance. As reported earlier (1), the activity increased progressively with increasing size of the alkyl group in this position, the 3-*n*-butyl-1-dimethylaminoethoxy derivative being the most active compound in the original group. We have now increased the size of the 3-substituent to *n*-amyl and *n*-hexyl (compounds 13 and 15) without significantly increasing the activity. Further modifications of this group, such as branching or the introduction of aryl or aralkyl groups (compounds 14, 16, and 17), greatly lowered the local anesthetic activity.

Alterations of the basic side chain, such as lengthening the chain, replacing the tertiary amine function with a primary or secondary amine group, or otherwise changing the nature of the terminal tertiary amino group, also had an adverse effect on anesthetic activity. In general, the dimethylaminoethoxy side chain was the most effective of the nonheterocyclic structures, as may be seen by an examination of Table II. It was also observed that replacement of the oxygen ether linkage with either sulfur or nitrogen (compounds 29 and 33) lowered the activity.

A number of piperidyloxy derivatives (Table III) with the desirable 3-*n*-butyl side chain were quite effective topically. For the most part, however, these compounds were somewhat more toxic and showed irritation at higher concentrations. One notable exception is 1-[3-(1-methylpiperidyloxy)]-3-*n*-butylisoquinoline (compound 40) which produced anesthesia lasting for 155 minutes at a concentration of 0.001% with no irritation noted at concentrations as high as 0.1%.

The introduction of alkyl, alkoxy, amino, acetamido, or halogen groups into the isoquinoline nucleus lowered the activity (Table IV) and increased toxicity and irritation.

SUMMARY

1. A series of isoquinoline derivatives has been prepared and tested for topical anesthesia by application to the cornea of rabbits.

2. Isoquinoline compounds with the *n*-butyl group in the 3-position and a piperidyloxy side chain attached through the 1-position were found to be the most active. One of these, 1-[3-(1-methylpiperidyloxy)]-3-*n*-butylisoquinoline was outstanding in this respect.

3. A new synthesis of substituted isoquinolines is described.

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WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal, and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

Alloocimene
Ecgonine
Fainesene
Hydrocoerulignone
Melanin
Ocimene
Scopine
Tropine
Xanthotoxin
5-Nitrooxine
2-Hydroxynicotinic acid
2-Acetamido-1,4-naphthoquinone
3-Chloro-1-butanol
cis-Cinnamaldehyde
Ethylene diphthalimide
2,4,6-tris(Ethyleneimine)-*s*-triazine
α-Glycerolphosphate dehydrogenase
β-Naphthyl hydrazine hydrochloride
Tetramethylthionine
Taurocholic acid

A Comparative Study of Prantal and Banthine in Hyperhidrosis*

By ARTHUR G. ZUPKO and LEON D. PROKOP†

Quantitative determinations of sweat on various parts of the body were done prior to and after oral administration of Prantal, Banthine, or placebo tablets. A discussion is presented of the comparative effects of these drugs in reducing sweat in hidrose subjects and in normal subjects in whom hyperhidrosis was induced. The effects of prolonged Prantal administration on the hematopoietic system, cardiac mechanism, and basal metabolic rate of subjects with hyperhidrosis, as well as side reactions, are compared with a previously reported study on Banthine.

RECENTLY Sperber, *et al.* (1), described a new quaternary amine, Prantal (N, N-dimethyl-4-piperidylidene-1, 1-diphenylmethane methyl sulfate). Subsequent pharmacological study indicated that Prantal was an effective parasympathetic blocking agent with a more prolonged action and fewer side effects than Banthine (2). Clinical studies have shown that Prantal diminishes motility and secretions of the stomach in peptic ulcer patients with minimum side effects (3–5).

The effectiveness and side effects of Banthine in controlling hyperhidrosis have been reported previously (6, 7). The similarity in parasympathetic blocking action of Banthine and Prantal prompted Nelson (8) to compare the effectiveness of these quaternary amines in diminishing sweat secretions. Results in a small series of patients showed Prantal to be effective in controlling some cases of hyperhidrosis but neither Prantal nor Banthine was consistently effective. In order to evaluate further the efficacy of Prantal and Banthine, studies were undertaken to obtain quantitative data on their ability to diminish sweating. It seemed desirable to compare these drugs further by administering Prantal to a large group of hidrose subjects over a prolonged period of time and comparing the side effects as well as the effects on the hematopoietic system, cardiac mechanism, and basal metabolic rate with a previously reported study on Banthine (7).

EXPERIMENTAL

Methods.—Eighteen volunteer subjects with varying degrees of hyperhidrosis and 16 normal subjects were used as a test group for quantitative determinations of sweat on various parts of the body prior to and after drug or placebo administration. Hyperhidrosis was induced in normal subjects through mild exercise in the form of a small, measured amount of work. Hidrose subjects were exposed to elevated temperatures. A majority of the experiments were conducted in a constant temperature room at consistently high dry bulb temperatures (85–95° F.) with variations in relative humidity (40–85%). For the collection of sweat, a modification of Randall's method (9) was used. The bottom half of a ½-oz. ointment tin containing activated alumina was covered with an absorbent blotting paper disk cut to fit precisely into the tin. The upper half or cover was also fitted with a similar disk. This unit could be heated in an oven and stored in an activated alumina desiccator. Immediately before use the prepared ointment tin was weighed on a precision Chain-O-Matic balance. The cover with its absorbent disk was quickly removed and stored in the desiccator while the capsule was inverted, covered with aluminum foil, and taped securely over the test area. Sweat counts were done simultaneously, using the dichlorofluorescein method and Wood's light as described by Sulzberger, *et al.* (10), and the iodine-impregnated starch paper method of Thaysen (11). At the end of the test period the tin was removed, and the blotter disk from the desiccator was applied over the test area to pick up any unabsorbed sweat. The entire unit was then weighed immediately. The change in weight of the prepared tin represented the amount of sweat secreted during the test period. Blank tins were run simultaneously to correct for errors induced by variations in environmental temperature and humidity. Rubber finger cots were used by the operators during the course of the experiments.

Forty-two volunteer subjects with varying degrees of hyperhidrosis and eight normal subjects were used to observe the effects of Prantal on the hematopoietic system, cardiac mechanism, and basal metabolism. All the subjects were below forty-five years of age with an average age of 25.4 years. The study included 13 females and 37 males. Of these, 8 were Negroes (6 female and 2 male). One of the females was in the third month of pregnancy and a second in the fifth month. The routine used was essentially the same as reported in a previous study with Banthine (7). In mild cases, two 100-mg. tablets were taken either a. c. or p. c. twice a day. In the more severe cases, the dosage was increased proportionately but never exceeded 800 mg. per day. The eight normal subjects were given 100 mg. of the drug in the morning and 100 mg. in the evening. Both

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TABLE I.—A TYPICAL CHART SHOWING THE EFFECT OF 100 MG. OF BANTHINE ON A HIDROSE SUBJECT AT 91° F. TEMPERATURE AND 61 PER CENT HUMIDITY

Area	Normal Sweating at Elevated Temperature			Sweating after Banthine Administration at Elevated Temperature					
				30 Min.			60 Min.		
	Sweat Collected, Mg./Sq. Cm./Min.	Av. No. Active Glands per Sq. Cm.	Av. Output per Gland, Mg./Min.	Sweat Collected, Mg./Sq. Cm./Min.	Av. No. Active Glands per Sq. Cm.	Av. Output per Gland, Mg./Min.	Sweat Collected, Mg./Sq. Cm./Min.	Av. No. Active Glands per Sq. Cm.	Av. Output per Gland, Mg./Min.
Forehead	1.343	219	0.0061	1.284	121	0.0107	0.991	57	0.0174
Dorsum of foot	0.368	101	0.0036	0.337	46	0.0073	0.283	39	0.0073
Forearm	0.274	46	0.0059	0.281	33	0.0085	0.256	31	0.1220

groups of subjects continued the medication for three to five months.

RESULTS

Quantitative Estimation of Sweat.—Only male subjects were used in the series of experiments described in the following:

Banthine.—Nine hidrose subjects were exposed to elevated temperatures in a constant temperature room for a period of thirty minutes. Prepared ointment tins were placed on various body parts prior to exposure. During the exposure, sweat patterns were taken at two-minute intervals and the number of active glands were noted. At the end of the thirty-minute period, the prepared tins were removed and immediately weighed, the amount of sweat secreted being carefully recorded.

Banthine bromide (100 mg.) was then administered, and the process was repeated after an additional thirty and sixty minutes. Charts were maintained for each subject showing the sweat collected, the average number of active sweat glands, and the output per gland before and after the drug administration. A typical chart is shown in Table I.

Eight normal subjects performed a small, measured amount of work in mildly elevated temperatures to induce hyperhidrosis. The same procedure as outlined above was used, with the exception that performed work was repeated at the beginning of the second thirty-minute period, at the time of Banthine administration.

Prantal.—Nine hidrose and 8 normal subjects were tested in a manner similar to that described for Banthine, with the exceptions that Prantal was administered in 200-mg. doses and the subjects were tested for 120 minutes. The thirty-minute testing periods were adhered to in order to take into account all initial, progressive, and delayed responses of the sweat glands.

Results indicate that normal and hidrose subjects react somewhat similarly to administration of Prantal and Banthine. Table II shows the comparative effect of these drugs in reducing sweat on various parts of the body in the subjects. From this table it will be noted that Prantal is comparatively ineffective in diminishing sweat during the first thirty minutes, whereas Banthine reduces sweat approximately 10% during this same period. After sixty minutes, Prantal reduces sweat slightly less than 10%. After this same period of time, Banthine reaches its peak of effectiveness, but beyond this sixty-minute period Banthine does not reduce sweat appreciably. Prantal reaches a point of effectiveness somewhere between sixty and ninety minutes, with a peak of effectiveness at between ninety and 120 minutes. In four areas (dorsum of hand, forearm, forehead, dorsum of foot) Banthine seems to be more effective than Prantal in reducing sweat under the experimental conditions stated. Prantal seems to be more effective in 3 areas (upper arm, leg, and thigh). The effectiveness of the drugs on various body parts may conceivably vary greatly with a larger group of subjects.

A few selected normal and hidrose individuals were subjected to the procedure described above with the exception that they were given placebos. Reduction of sweat at the conclusion of the experi-

TABLE II.—EFFECT OF PRANTAL AND BANTHINE ON PERCENTAGE REDUCTION OF SWEAT IN NORMAL AND HIDROSE SUBJECTS

Area	Banthine		Prantal			
	30 Min.	60 Min.	30 Min.	60 Min.	90 Min.	120 Min.
Dorsum of hand	11.4[a] (0.3–22.1)	26.2 (7.6–48.3)	0.3 (0.0–4.7)	3.4 (0.6–18.4)	21.7 (6.9–31.7)	19.4 (8.7–28.3)
Forearm	6.7 (0.0–11.5)	31.4 (3.6–38.9)	1.1 (0.0–5.2)	7.3 (1.8–21.2)	16.9 (8.7–30.1)	17.7 (11.3–34.6)
Upper arm	4.8 (0.2–19.4)	18.7 (3.2–41.3)	0.8 (0.0–3.4)	9.9 (4.1–21.2)	23.6 (11.7–39.8)	21.5 (10.1–40.0)
Forehead	19.6 (2.1–41.2)	42.5 (19.3–58.4)	2.7 (0.0–6.9)	11.0 (2.3–24.7)	37.7 (18.4–40.3)	38.2 (21.1–47.4)
Dorsum of foot	12.3 (1.7–39.8)	36.8 (17.9–51.2)	1.8 (0.0–4.1)	6.9 (1.4–21.8)	29.1 (19.3–41.7)	26.3 (17.4–50.1)
Leg	8.4 (2.1–27.6)	21.7 (9.1–38.3)	0.9 (0.0–2.7)	12.7 (3.9–27.3)	28.0 (18.4–47.1)	29.7 (20.0–51.7)
Thigh	10.5 (1.8–31.9)	26.9 (16.6–41.4)	2.9 (0.0–6.1)	9.4 (2.3–22.5)	20.7 (11.1–36.7)	28.3 (22.4–54.1)

[a] Values not in parentheses represent average percentage reduction in all subjects. Figures in parentheses represent the range in percentage reduction obtained.

ments ranged from 2.1 to 6.7% in 3 subjects but was increased from 1.8 to 5.4% in two others.

Sweat pattern tests show that with exercise or exposure to elevated temperatures there is at the onset a prompt increase in the number of functioning glands with a concomitant increase in sweat collected from the area.

The calculated output per gland does not necessarily increase, however. With continued exposure there is an increase in output per gland but not necessarily an increase in the number of active glands. These observations are in agreement with those reported by Randall in his study of the output of individual sweat glands (9). Both Prantal and Banthine significantly reduced the number of actively functioning sweat glands, as well as the output per gland.

Effect on Hyperhidrosis.—Forty-two hidrose subjects were arbitrarily classified as individuals with mild, pronounced, or severe hyperhidrosis, depending upon the severity of the symptoms.

Prantal in 200-mg. doses twice a day effectively controlled sweating in 20 of 23 subjects classified as mild cases. Doses up to 600 mg. daily effectively controlled sweating in 12 of 16 subjects classified as pronounced, and with doses up to 800 mg. daily, 1 of 3 severe cases was effectively controlled. Prantal controlled hyperhidrosis in 33 of 42 subjects or 78.6% when given in 400- to 800-mg. daily doses over a period of three to five months.

Of the 9 subjects in whom Prantal was ineffective, 5 were effectively controlled by 150- to 200-mg. daily doses of Banthine. Four subjects were not affected by either Prantal (in daily doses up to 800 mg.) or Banthine (in daily doses up to 200 mg.).

Side Effects.—Prantal administration was not completely devoid of side effects, as noted in Table III. Side effects were reported by 2 controls and 17 different hidrose individuals, several of whom reported more than one side effect. In all instances, except one, the side effects were mild or disappeared within a short time. Persistent headache necessitated discontinuance of the drug in the instance cited above. It is interesting to note that 3 subjects complained of diarrhea and gastric irritation with Prantal, whereas in a previously reported study with Banthine (7), 18.8% of the subjects complained of constipation. Xerostomia was reported by every subject taking Banthine, but only 11 subjects taking Prantal complained of this side effect. Headaches and mydriasis were reported less frequently with Prantal than with Banthine. Prantal did not cause heartburn or dysuria in any of the subjects, but these side effects were reported with Banthine.

Effect on Cardiac Mechanism.—The ingestion of Prantal did not significantly alter the rate or rhythm of the heart at the dosage levels necessary to control hyperhidrosis in the subjects under study. One subject reported an arrhythmia as a result of Prantal ingestion, but 3 subsequent electrocardiogram tracings did not substantiate this.

Effect on the Hematopoietic System.—All the subjects under study were within normal hematological limits before and after Prantal administration with two exceptions. These 2 subjects had a hypochromic anemia and were under a physician's care. As with Banthine, the administration of Prantal did not interfere with the expected improvement in the subjects under treatment for anemia.

Effect on Basal Metabolism.—No appreciable change was noted in either the hidrose or control subjects after many weeks of Prantal administration.

TABLE III.—SIDE EFFECTS AFTER PRANTAL

Side Effect	Number	Per cent
Headache	4	8.0
Mydriasis	2	4.0
Diarrhea	3	6.0
Xerostomia	11	22.0
Gastric irritation	3	6.0
Drowsiness	2	4.0

DISCUSSION

Subjects with complaints of side effects were given placebos simulating the appearance and taste of Prantal. Invariably relief from the side effects was reported. A few subjects claimed relief from hyperhidrosis with placebos, necessitating their being dropped from the test groups, since it was felt their subjective reports were unreliable. Generally, placebo administration substantiated the subjective reports of the test group.

The duration of activity of both Prantal and Banthine was approximately two and one-half to four and one-half hours.

Sixteen subjects were tested to determine the advisability of giving Prantal before or after meals. All subjects reported that the best results were noticed when Prantal was ingested about one hour before a meal. This is in accordance with the results reported in Table II.

Administration of Prantal must be continued indefinitely for effective control of hyperhidrosis, as evidenced by the fact that when the drug was discontinued the excessive sweating appeared with regularity.

Daily administration of the drug over a period of many weeks had no effect upon either menses or pregnancy in the female subjects under study. The Negro and Caucasion subjects did not show any significant differences in their response to Prantal. Tolerance to Prantal was not reported by any of the subjects.

TABLE IV.—COMPARATIVE EFFECTS OF PRANTAL AND BANTHINE

Effect	Percentage of Subjects Reporting Effect	
	Prantal	Banthine*
Effective control of hyperhidrosis	78.6	90.9
Constipation	None reported	18.8
Mydriasis	4.0	6.3
Dysuria	None reported	4.2
Headache	8.0	27.1
Diarrhea	6.0	None reported
Heartburn	None reported	16.7
Xerostomia	22.0	100.0
Drowsiness	4.0	Unknown
Tolerance	None reported	10.0

* Figures obtained from a previously reported study on Banthine (7).

The comparative effects of Prantal and Banthine are summarized in Table IV.

SUMMARY

1. Neither Prantal nor Banthine is completely effective in controlling hyperhidrosis. In the present study 9 subjects failed to respond to Prantal treatment. Of these, 5 responded satisfactorily to Banthine, while 4 did not respond to either drug.

2. Banthine, although effectively controlling hyperhidrosis in approximately 90 per cent of the test subjects, has many undesirable side effects. Prantal checks hyperhidrosis with slightly less effectiveness, but apparently with considerably fewer and milder side effects.

3. Prantal becomeseffective between sixty and ninety minutes after administration while Banthine becomes effective in thirty minutes. The peak of effectiveness is reached in ninety to 120 minutes with Prantal and in only sixty minutes with Banthine.

4. The dosage of Prantal required to control hyperhidrosis is approximately twice that of Banthine.

5. None of the subjects taking Prantal seemed to develop a tolerance to the drug, but about 10 per cent of the subjects taking Banthine developed a tolerance.

6. Neither Prantal nor Banthine at the dosage levels necessary to control hyperhidrosis has any appreciable effect upon the cardiac mechanism, basal metabolism, or hematopoietic system of the subject.

7. Both drugs control hyperhidrosis in a majority of cases, but administration of the drugs must be continued indefinitely.

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The Effects of Surface-Active Agents on Bacitracin Activity *in Vitro*[*]

By C. B. BRUCE and L. MITCHELL

The antibacterial action of bacitracin is significantly and selectively affected by the presence of surface-active agents as determined by the paper-disk assay, using *Micrococcus flavus* as the test organism. Potency is enhanced by cationic and nonionic compounds and is decreased by anionic. This effect with benzethonium chloride can be shown in the case of other organisms, namely, *Staphylococcus aureus, Streptococcus pyogenes,* and *Neisseria catarrhalis.* Because of the potent antiseptic action of the quaternary ammonium agents, it is suggested that combinations of these with bacitracin may be well suited to the local treatment of infections where greater penetration of the tissue is desired.

IT HAS BEEN SHOWN that the activity of many antiseptics can be increased by the addition of surface-active agents (1) and that the latter themselves often posses bacteriostatic or bactericidal properties (2). Even when the antibacterial activity of the surface-active agent is relatively weak or nonexistent, this enhancement has been demonstrated, for example, by Youmans (3) in the case of Tween 80 (a nonionic detergent) with some 20 agents, including chloramphenicol, against *Mycobacterium tuberculosis in vitro.* Gregg and Zopf (4) have recently shown that Tween 80 greatly enhances the bactericidal power of hexachlorophene *in vitro.* Grace and Bryson (5) combined sodium tetradecyl sulfate (an anionic detergent) with penicillin, and found definite advantages over the antibiotic alone in the local treatment of infected bone and soft tissue.

Of the various types of surface-active agents, the cationic (particularly the quaternary ammon-

* Received November 29, 1951, from the Research Laboratories, Frank W. Horner, Ltd., Montreal, Canada.

ium germicides) probably have the greatest antiseptic power. It seemed desirable, therefore, to test combinations of these agents with certain antibiotics for topical use where increased antiseptic action and more effective contact with tissue surfaces might have practical advantage. However, when a quaternary ammonium agent (benzethonium chloride) was combined with tyrothricin, it was found that the bactericidal effect of the latter against *Streptococcus fecalis in vitro* was markedly antagonized.[1] Accordingly, a more thorough investigation of another polypeptidic antibiotic, bacitracin, was undertaken with representatives of cationic, anionic, and nonionic surface-active agents in order to study the question of enhancement or antagonism.

EXPERIMENTAL

The surface-active agents tested were the quaternary ammonium compounds benzethonium chloride (N. N. R.) and B. T. C.[2] (cationic); Duponol C[3] and Tergitol-4[4] (anionic); Tween 80[5] and Tergitol N. P. X.[6] (nonionic). Solutions were prepared containing 10, 20, and 40 μg./cc. of these agents. Similar solutions, containing also 1 unit/cc. of bacitracin,[7] were prepared. For comparison, a bacitracin solution of 1 unit/cc. was used throughout.

The assay was similar to the F. D. A. method for bacitracin (6), using *Micrococcus flavus* as the test organism in 1% agar. Paper disks were used in place of cups[8] and so necessitated somewhat higher concentration of the antibiotic in order to obtain zones of inhibition of suitable diameter. A standard slope was made, using concentrations of bacitracin ranging from 0.5 to 5.0 units/cc. All readings were done with a Fisher-Lilly Antibiotic Zone Reader.

The standard bacitracin solution of 1 unit/cc., the solutions of surface-active agents, and the corresponding solutions of bacitracin plus surface-active agents were applied in diametrically opposite pairs to each plate; 5 plates were used at each of the 3 concentrations of the 6 surface-active agents. After incubation, the plates were read and adjustments were made for discrepancies between the individual plate readings and the standard slope as directed (6). Standard errors were calculated after these corrections were applied. Results expressed as percentage activity of standard bacitracin are listed in Table I.

An additional experiment was undertaken to see if any of these effects extend to other organisms, *viz.*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Neisseria catarrhalis*. In order that all organisms could be grown on a common medium, a mixture of brain heart infusion and tryptose phosphate broth (Difco) with 1% agar was used as a seed layer. Twenty-four-hour broth cultures of the organisms were used, and the plates were read after eighteen hrs. at 37°, using essentially the same technique as before. Bacitracin was tested at a concentration of 10 units/cc. alone and in combination with 100, 200, and 400 μg./cc. of benzethonium chloride. In these experiments no standard slope for the antibiotic against individual organisms was made.

The results given in Table II are expressed simply as the means of the zones of inhibition with the standard errors. The zone for each combination of bacitracin and surface-active agent is shown, together with the average value for the bacitracin control on those particular plates. The significance of the difference between these values is given in the last column, expressed as x/σ.

TABLE I.—RELATIVE POTENCIES OF BACITRACIN—S. A. A. COMBINATIONS AGAINST MICROCOCCUS FLAVUS

Surface-Active Agent (S. A. A.)	Type	Ratio of S. A. A.: Bacitracin[a]	Relative Potency[b]
Benzethonium chloride	Cationic (Quat. NH_4)	10:1	132 ± 3.6
		20:1	142 ± 1.5
		40:1	146 ± 2.4
B. T. C.	Cationic (Quat. NH_4)	10:1	134 ± 2.4
		20:1	141 ± 3.9
		40:1	148 ± 5.4
Tween 80	Nonionic	10:1	129 ± 1.5
		20:1	132 ± 3.5
		40:1	135 ± 5.2
Tergitol N. P. X.	Nonionic	10:1	133 ± 2.7
		20:1	138 ± 6.8
		40:1	145 ± 2.2
Duponol C	Anionic	10:1	62 ± 2.0
		20:1	46 ± 2.8
		40:1	43 ± 3.0
Tergitol 4	Anionic	10:1	87 ± 12.1
		20:1	84 ± 6.9
		40:1	63 ± 2.3

[a] Where the S. A. A. is expressed as μg./cc. and bacitracin as units/cc.
[b] Standard bacitracin = 100.

DISCUSSION

From Table I it is seen that in the presence of the anionic wetting agents, bacitracin is partially inactivated or antagonized while the nonionic and cationic compounds significantly enhance its bacteriostatic activity against *Micrococcus flavus*. Although statistical calculation sometimes fails to establish significantly increased activity with increasing concentration of the surface-active agent, it can be taken that this is the trend in the present experiperiments. None of the surface-active agents alone demonstrated any significant antiseptic activity in the concentrations and conditions used in these tests. This is seen quite clearly in Fig. 1 which shows no inhibition with benzethonium chloride alone, while the combination with bacitracin is superior to the antibiotic alone. Presumably their contribution is to effect greater penetration of the antibiotic through the medium. This is an interesting feature in the case of quaternary ammonium compounds which are known to be potent bacteri-

[1] Unpublished results.
[2] Trade mark of Onyx Oil & Chemical Co. for alkyldimethylbenzylammonium chloride.
[3] Trade mark of I. E. du Pont de Nemours & Co., Inc., for sodium lauryl sulfate.
[4] Trade mark of Carbide & Carbon Chemical Corp. for sodium sulfate of 7-ethyl-2-methylundecanol-2.
[5] Trade mark of Atlas Powder Co. for polyethylene sorbitan monooleate.
[6] Trade mark of Carbide & Carbon Corp. for alkyl phenyl polyglycol ether.
[7] S. B. Penick & Co., 42 units/mg.
[8] The experiments with benzethonium chloride have since been repeated using cups, with the same results.

TABLE II.—ZONES OF INHIBITION WITH BACITRACIN AND COMBINATIONS WITH SURFACE-ACTIVE AGENTS

Test Organism	Ratio of S. A. A.: Bacitracin[a]	Width of Zone, Mm.	Significance of Difference from Control (x/σ)
Staphylococcus aureus	100:10	18.8 ± 0.26	5
	Control[b]	17.2 ± 0.20	
	200:10	21.4 ± 0.23	13
	Control	17.7 ± 0.17	
	400:10	25.5 ± 0.11	20
	Control	18.2 ± 0.20	
Neisseria catarrhalis	100:10	27.3 ± 0.95	0.6
	Control	26.5 ± 0.85	
	200:10	28.8 ± 0.76	1.0
	Control	27.6 ± 0.81	
	400:10	28.9 ± 0.22	3.5
	Control	27.4 ± 0.37	
Streptococcus pyogenes	100:10	42.1 ± 0.40	2.5
	Control	41.0 ± 0.22	
	200:10	42.9 ± 0.39	4.1
	Control	41.1 ± 0.20	
	400:10	43.5 ± 0.48	4.0
	Control	41.2 ± 0.33	

[a] Where the S. A. A. (surface-active agent) is expressed as μg./cc. and bacitracin as units/cc.
[b] Bacitracin control solutions tested at 10 units/cc.

cides. It is possible, therefore, that in more favorable media these agents would not only permit greater penetration of the antibiotic but also would exert antibacterial effects themselves against susceptible organisms. In the case of the nonionic agents which are known to be inactive or weak as antiseptics, enhanced penetration by the antibiotic is the only effect to be expected. However, this alone might be of practical worth since bacitracin has a diffusion coefficient only $^1/_3$ to $^1/_2$ that of penicillin (7).

It appears from the results in Table II that benzethonium chloride enhances the action of bacitracin against *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Neisseria catarrhalis* as well, although statistical calculation fails to disclose a significant effect at the lower ratios of the combination compared to bacitracin alone in the case of *Neisseria catarrhalis*.

In the case of *Staphylococcus aureus* it was noted that bacitracin alone always produced a hazy perimeter which microscopic examination showed to be due to the persistence of some sub-surface colonies. This was not noted whenever the surface-active agent was present, suggesting increased penetration of the antiseptic agent into the medium.

CONCLUSIONS

Bacitracin has been tested in combination with 6 surface active agents against *Micrococcus flavus in vitro*. The results appear to fall into two categories, (*a*) antagonism by anionic agents, and (*b*) enhancement by the cationic and nonionic agents. It has been shown that this exhancement extends to other organisms also, *viz.*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Neisseria catarrhalis*. Presumably this is due, in part at least, to superior penetration of the antibiotic through the medium. Since the cationic quaternary ammonium compounds possess strong antiseptic properties themselves, it is possible that their combinations with bacitracin might have practical application in the local treatment of infection.

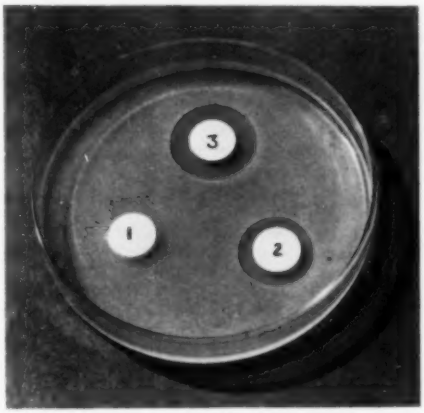

Fig. 1.—Photograph of comparative zones of inhibition on *Micrococcus flavus* produced by:

(1) benzethonium chloride alone, 10 μg./cc.
(2) bacitracin alone, 1 unit/cc.
(3) a combination of benzethonium chloride, 10 μg./cc., with bacitracin, 1 unit/cc.

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Routes to the Synthesis of 6-Amino-2-Naphthoic Acid*,†,‡

By G. STAPLETON§ and A. I. WHITE

Two new methods for the synthesis of 6-amino-2-naphthoic acid have been described. One of these, i. e., by way of 6-bromo-2-naphthylamine, produces the compound conveniently and in satisfactory quantities. The other method, i. e., by way of 6-nitro-2-naphthylamine, provides a procedure easily carried out but less satisfactory because of the small yield of 6-nitro-2-naphthylamine upon nitration of 2-naphthylamine.

THE SYNTHESIS of 6-amino-2-naphthoic acid was investigated because this compound was needed as starting material for the synthesis of substances hoped to be local anesthetics possessing vasopressor properties.

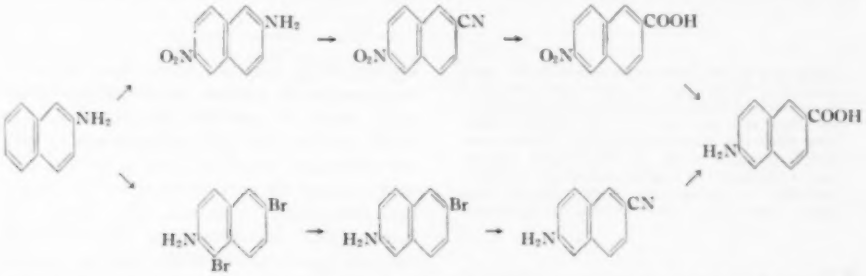
Harrison and Royle (1) have prepared 6-amino-2-naphthoic acid by the Bucherer reaction from 6-hydroxy-2-naphthoic acid which in turn had been prepared from 2-naphthylamine-6-sulfonic acid. Dziewonski, Schoenowna, and Waldmann (2) synthesized the same acid by forming the 6-amino-2-methylnaphthalene from 6-hydroxy-2-methylnaphthalene by the Bucherer reaction and subsequent potassium permanganate oxidation to the 6-amino-2-naphthoic acid. Both of these methods are troublesome and produce relatively small over-all yields.

Using 2-naphthylamine as starting material, two new routes for synthesizing 6-amino-2-naphthoic acid have been developed. In the first method, 6-nitro-2-naphthylamine was prepared from 2-naphthylamine by the method of Saunders and Hamilton (3). The nitro compound was converted to 6-nitro-2-naphthoic acid by diazotization to the nitrile, followed by hydrolysis. The 6-nitro-2-naphthoic acid was then reduced to 6-amino-2-naphthoic acid.

The second and more convenient route involves the synthesis of 6-bromo-2-naphthylamine from 2-naphthylamine according to the methods of Claus and Philipson (4) and Franzen and Stauble (5). After acetylation, the bromo compound may be converted to the desired acid by way of the nitrile, followed by hydrolysis.

EXPERIMENTAL

6-Nitro-2-naphthonitrile.—The method employed in this synthesis followed that of Blicke, Parke, and Jenner (6) in which they prepared 5-nitro-2-naphthonitrile from 5-nitro-2-naphthylamine. From 5.8 Gm. of 6-nitro-2-naphthylamine, prepared by the method of Saunders and Hamilton (3) using 2-naphthylamine, 5.1 Gm. of 6-nitro-2-naphthonitrile was obtained (83%). The brownish-yellow powder was insoluble in water, ethanol, benzene, purified petroleum benzin, and ether. It was slightly soluble in acetone, soluble in hot glacial acetic acid, and soluble in warm chloroform in which it produced a yellowish-green solution. It melted at 235–237° (uncorr.) with some decomposition beginning at 200°.



Anal.—Calcd. for $C_{11}H_6O_2N_2$: N, 14.14. Found: N, 14.23.

6-Nitro-2-naphthoic Acid.—The method used in this synthesis was adapted from that of Blicke, Parke, and Jenner (6) in which they prepared 5-nitro-2-naphthoic acid from 5-nitro-2-naphthonitrile. Three grams of 6-nitro-2-naphthonitrile hydrolyzed in 80% acetic acid with sulfuric acid yielded 2.6 Gm. of 6-nitro-2-naphthoic acid (79%).

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§ Fellow of the American Foundation for Pharmaceutical Education.

It melted at 320° (uncorr.) with some decomposition beginning at 285°. Harrison and Royle (1) report 6-nitro-2-naphthoic acid as melting at 310°.

6-Amino-2-naphthoic Acid.—Two grams of 6-nitro-2-naphthoic acid was suspended in 35 cc. of glacial acetic acid and 7 Gm. of powdered stannous chloride dihydrate was added. The mixture was stirred until the tin salt had gone into solution. A rapid stream of dry hydrogen chloride was then passed through the mixture and the temperature was maintained at 40–50°. After complete saturation with HCl, the mixture was allowed to stand at room temperature for six hours. The reaction mixture was added to 200 cc. of distilled water, and the insoluble material was filtered off. The filtrate was neutralized with 10% sodium hydroxide until a precipitate formed. After cooling, the precipitate was collected and then recrystalized from ethanol and water. The yield was 1.2 Gm. (70%). It melted at 227° (uncorr.). Harrison and Royle (1) reported 6-amino-2-naphthoic acid as melting at 225°.

6-Acetamido-2-naphthonitrile.—In a 250-ml. one-necked ground glass flask fitted with a reflux condenser were placed 26.7 Gm. (0.1 mole) of 6-bromo-2-acetamidonaphthalene (5), 10.7 Gm. (0.12 mole) of cuprous cyanide, and 20 cc. of dried redistilled pyridine, according to the method of Newman (7) for the preparation of 1-naphthonitrile. The mixture was refluxed for twenty-two hours. At the end of the refluxing period, there was a solid mass in the bottom of the flask which was removed, pulverized, and washed with ammonium hydroxide. The solid residue was then heated with successive portions of alcohol until most of it had dissolved. The alcoholic solutions were combined and heated to boiling, and dilute ammonia water was added to the point of cloudiness. On cooling, 12.7 Gm. (60%) of silver-colored needles was obtained, melting at 263–265° (uncorr.).

6-Amino-2-naphthoic Acid.—A quantity of 10.5 Gm. (0.05 mole) of 6-acetamido-2-naphthonitrile was refluxed with a mixture of 20 cc. of sulfuric acid, 20 cc. of glacial acetic acid, and 20 cc. of water for two hours. The reaction mixture was then poured into 200 cc. of distilled water and allowed to cool. The precipitate which formed was filtered off and dissolved in a solution of sodium carbonate with the aid of heat, filtered, and carefully neutralized with acetic acid. The 6-amino-2-naphthoic acid precipitated from the neutral solution and was separated by filtration. After crystallization from ethanol and water, the yield was 7.8 Gm. (69%) of light tan needles melting at 227–228° (uncorr.).

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The Effect of Phenergan on Tetanus Spore Infection*

By LOUIS GERSHENFELD,† JOSEPH W. E. HARRISSON,‡ and ARTHUR E. GREENE†

Although it had been reported that the antihistamine Phenergan increases the spread of bacterial and viral infections in the host, such an effect was not observed in tetanus infection after the administration of Phenergan. Antihistamines such as Phenergan appear not to produce any marked increase in tetanus fatalities or symptoms, even though they cause spreading of the microorganisms.

HALPERN (1) observed that the antihistamine, Phenergan, prevented the appearance of the local inflammatory reaction normally produced in rabbits by the hypodermic injection of *Salmonella typhimurium*. At the same time, however, septicemia with positive blood cultures developed; hence, the mortality rate increased. The ability of Phenergan to lessen resistance to various infections, including those caused by normally saprophytic organisms and by viruses, has been repeatedly confirmed (2). Barski (3) demonstrated that cutaneous infections with vaccinia virus in rabbits tend to become generalized if the animals are treated with Phenergan; most rabbits died from viral encephalitis, while the untreated infected animals all survived. Rose and Rose (4) showed that Pyribenzamine is able to break down the resistance of newts toward Lucké kidney adenocarcinoma which cannot be transplanted to untreated newts. On the other hand, Weeks and Gunnar (5) studied in detail the microscopic appearance of inflammatory reac-

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tions after the administration of the antihistamine Pyribenzamine in rabbits and found no significant effects of the latter drug. In view of these facts, we were interested in testing the effects of Phenergan on infections with tetanus spores. Phenergan was chosen as the antihistaminic agent since this substance exhibits strong and long-lasting activity.

METHODS

The mice were divided into 2 groups. One group was used as a control and did not receive Phenergan. The experimental group received 10 mg./Kg. of Phenergan subcutaneously. The experimental and control groups each contained 18 mice.

Concentrated spores were diluted 1:90 in 3% $CaCl_2$ solution. Each 0.1 ml. of this 1:90 dilution contained 7.6×10^3 spores. Both groups of mice were given 0.1 ml. of spores (1:90 dilution) in 3% $CaCl_2$ solution in the right flank subcutaneously.

The experimental group was given 10 mg./Kg. of Phenergan in the left flank approximately two hours before the spore injection. The control group did not receive the antihistamine. The experimental group was subsequently given 10 mg./Kg. of Phenergan daily until the end of the experiment.

RESULTS

From the data in Table I, it is apparent that Phenergan does not increase sensitization of mice to tetanus infection caused by injections of spores in 3% $CaCl_2$ solution.

DISCUSSION

The method of action of antihistamines on infectious processes is not known. Many authors have suggested that this activity is due to the antagonistic action toward inflammation and phagocytosis. The inhibitory effect of antihistamines on inflammation suggested by some workers does not seem to hold true according to the work of Weeks and Gunnar (5). The second action, however, is supported by the findings of Jancso (6), Matoltsy, *et al.* (7), and Biozzi, *et al.* (8), who showed that histamine increases the phagocytic activity of the reticuloendothelial system, while antihistamines decrease this effect. The histological picture of the infected tissue after the injection of tetanus spores was presented by Fildes (9). According to his data, local inflammation and phagocytosis play a small part in tentanus infection, since even a small number of bacteria elaborate enough toxin to kill the host. This may explain the failure of Phenergan to enhance tetanus infection in mice.

TABLE I.—THE EFFECT OF PHENERGAN UPON TETANUS SPORE INFECTION IN MICE[a]

Experimental Group	No. of Mice Used	Death with Tetanus Symptoms	Survivors Exhibiting Tetanus Symptoms	No Symptoms
Phenergan, 10 mg./Kg. daily	18	11	2	5
Controls	18	10	3	5

[a] Mice injected subcutaneously with 7.6×10^3 spores in 3% $CaCl_2$ solution.

SUMMARY

1. Two groups of male Webster mice were injected subcutaneously with tetanus spores in 3% $CaCl_2$ solution. One group received 10 mg./Kg. of Phenergan subcutaneously two hours previous to the spore injection; the other group did not receive the antihistamine. The Phenergan treatment was continued daily for eight days.

2. Phenergan did not sensitize mice to tetanus spore infection.

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Extraction of Belladonna Root by Various Furans*,†

By E. D. CARKHUFF‡ and L. G. GRAMLING§

Data have been collected and tabulated on the Soxhlet extraction of belladonna root by furan, 2-methylfuran, tetrahydrofuran, and 2-methyltetrahydrofuran; and on the ability of the following furan azeotropes to extract the alkaloids of belladonna root: 2-methylfuran, 2-methyltetrahydrofuran, tetrahydrofuran-methanol, tetrahydrofuran-ethanol, and tetrahydrofuran (4.3% water). A procedure for the extraction of belladonna root with tetrahydrofuran azeotrope (4.3% water) by Soxhlet extraction is discussed. The isolation and identification of sucrose, malic acid, *l*-hyoscyamine sulfate, aurichloride, and picrate by the use of the above extraction is given.

IT WAS DESIRED to learn if certain furans would extract the alkaloids of belladonna root, using a Soxhlet extractor. It was felt that if hyoscyamine could be extracted in its natural form, racemization to atropine would not occur.

The furans used in this investigation included furan, 2-methylfuran, tetrahydrofuran, and 2-methyltetrahydrofuran (1). Hyoscyamine and atropine alkaloid were each found to be soluble to the extent of 0.1 Gm. in 0.4 cc. of tetrahydrofuran, in 1.1 cc. of 2-methyltetrahydrofuran, in 0.5 cc. of furan, and in 2.0 cc. of 2-methylfuran, respectively.

Atropa belladonna root, as the crude drug, was purchased from a reputable manufacturer in the standard coarse powder. The same lot of belladonna root was used through the entire investigation and was assayed for alkaloidal content by the N. F. IX method. Moisture content was determined by the U. S. P. XIV toluene method. The alkaloidal content of the drug was found to be 0.47 per cent and the moisture averaged 9.0 per cent.

EXPERIMENTAL

Extraction Procedure.—All furans and furan azeotropes used (1) were prepared by distillation.

The general procedure employed in the furan and furan azeotrope extraction of belladonna was as follows:

Accurately weighed samples of belladonna root were transferred to Soxhlet extractors and macerated. The extraction was conducted at a reflux of approximately 40 cc. per five minutes for the time indicated. At the expiration of this period, the extraction was interrupted and the extracted drug was removed and allowed to air dry.

The marc was then assayed for alkaloidal content by the N. F. IX method. The percentage of alkaloid extracted by the particular furan and furan azeotrope was then calculated. The total extractive was determined after vacuum desiccation over magnesium perchlorate. Results are shown in Table I.

Tetrahydrofuran Azeotrope (4.3% Water) Extraction.—Accurately weighed samples (50 Gm.) of belladonna root were placed in Soxhlet extractors. The distilling pots (250 cc.) were filled with 100 cc. of pure tetrahydrofuran and 30 cc. of tetrahydrofuran azeotrope (1). The drug was moistened with sufficient tetrahydrofuran azeotrope to wet it completely and cause siphoning. Extraction was begun and continued for four hours at a reflux of approximately 40 cc. per five minutes.

After refluxing for four hours, the extraction was interrupted, just after siphoning, long enough to add a fresh thimble containing 50 Gm. of drug to the Soxhlet extractor. The drug was moistened and extracted with tetrahydrofuran azeotrope as was the preceding sample.

During the course of extraction, crystals of sucrose formed in the boiling solvent. With the completion of the extraction, the flasks were set aside overnight to allow complete crystallization. The sucrose was filtered off the following morning, the flasks were washed out with tetrahydrofuran azeotrope, and the washings were passed through the filter. The filtrate was reserved for the isolation of *l*-hyoscyamine sulfate, aurichloride, and picrate.

Isolation of Sucrose.—The crystals of sucrose so obtained were dissolved in water and the solution was decolorized with charcoal. The water was evaporated to approximately 2 cc. and crystallization of the sucrose was induced by the addition of ethanol. The crystals were filtered from the mother liquor and dried in vacuum over magnesium perchlorate. The percentage of sucrose in belladonna root was calculated to be 3.12%.

The identity of the sucrose was established in the following manner:

The melting point of the crystals was observed to be 179–181° and a mixed melting point determination with C. P. sucrose gave 179–181°.

The specific rotation of the crystals in aqueous solution was found to be $[\alpha]_D^{25}$ +66.47. The specific rotation of sucrose is given as $[\alpha]_D^{25}$ +66.4 (2).

***l*-Hyoscyamine Sulfate.**—The tetrahydrofuran azeotrope from which the sucrose had been filtered was removed by distillation, leaving the residue which contained the alkaloids of belladonna root. This residue was treated with 20 cc. of water and

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† Based on a dissertation presented to the Graduate Council of the University of Florida by E. D. Carkhuff in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
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TABLE I.—EXTRACTION OF BELLADONNA ROOT BY VARIOUS FURANS

Solvent	B. P.	Maceration, Hr.	Extracted, Hr.	Total Extractive, %	Alkaloid Extracted, %
Furan	33–3	1/4	4	0.66	38.3
2-Methylfuran	62–64	1/4	5	3.8	19.2
2-Methylfuran-water	58–59	1/4	5	4.3	23.9
2-Methyltetrahydrofuran	80	1/4	9	3.9	58.0
2-Methyltetrahydrofuran	80	12	9	3.9	59.0
2-Methyltetrahydrofuran	80	12	12	4.1	71.8
2-Methyltetrahydrofuran-water	72–73	1/4	5	3.3	67.0
Tetrahydrofuran	65–4	1/4	10	3.8	47.1
Tetrahydrofuran-methanol	60–61	1/4	4	3.8	56.3
Tetrahydrofuran-ethanol	70–72	1/4	5	4.5	94.7
Tetrahydrofuran-water	63–4	1/4	3 1/2	11.3	92.5
Tetrahydrofuran-water	63–4	1/2	4	11.3	92.5
Tetrahydrofuran-water	63–4	1/4	4 1/2	11.3	92.5
Tetrahydrofuran-water	63–4	1/4	5	11.3	92.5
Tetrahydrofuran-water	63–4	1/4	6	11.3	92.5

gently heated. The aqueous mixture resulting from this treatment was extracted with three 30-cc. portions of ethyl ether, giving a clear, reddish, aqueous solution. The ether extracts were combined and tested for free alkaloid by the U. S. P. XIV method. At no time were alkaloids found to be in the ether extract. The aqueous solution gave definitely positive alkaloidal tests with alkaloidal reagents (3).

The aqueous solution was heated to expel dissolved ether, cooled, and extracted with three 20-cc. portions of chloroform. The aqueous portion was made alkaline by the dropwise addition of dilute ammonium hydroxide to *p*H 10 and was allowed to stand for ten minutes. It was then rapidly and thoroughly extracted by four 40-cc. portions of chloroform. The combined chloroform extractions were evaporated to dryness. The work of Carr and Reynolds (4), in an experiment with hyoscyamine *d*-camphorsulfonate dealing with optical rotation, indicate that hyoscyamine is not converted to atropine by this rapid treatment. The residue was treated with 10 cc. of water and broken up by a stirring rod. Then 0.5 *N* sulfuric acid was added dropwise with constant stirring to *p*H 5–6.

The water was evaporated by a jet of warm air and final drying by vacuum desiccation over magnesium perchlorate. The resulting alkaloidal sulfate was washed with tetrahydrofuran azeotrope and recrystallized from isopropyl alcohol. After drying at 105° for one hour, the melting point was determined to be 206°. Further recrystallization did not raise the melting point. A mixed melting point was found to be 206°. Henry (5) states the melting point of hyoscyamine sulfate to be 206°.

The specific rotation was determined to be $[\alpha]_D^{25}$ −32.64. Carr and Reynolds (4) give $[\alpha]_D^{25}$ −32.6 as the specific rotation for the basic ion as salt in water.

Hyoscyamine Aurichloride.—The residue obtained from the extraction of belladonna root by tetrahydrofuran azeotrope (see tetrahydrofuran azeotrope extraction) was treated with three 30-cc. portions of water and centrifuged. The aqueous phases were combined and thoroughly extracted with chloroform to remove any free alkaloid present. The aqueous solution was then made alkaline to *p*H 10 with ammonium hydroxide and rapidly extracted with four 30-cc. portions of chloroform. The combined chloroform extracts were evaporated and the residue was taken up in 30 cc. of 4% hydrochloric acid. To this acid solution was added 1:30 gold chloride solution dropwise until no further precipitation was noted. Then 5 cc. of the gold chloride solution was added in excess.

The crystals which formed after standing overnight were filtered off, washed with a small portion of water, and dried *in vacuo* over magnesium perchlorate.

The melting point was determined as being 165°. A mixed melting point with known hyoscyamine aurichloride was found to be 165°. Henry (5) reports the melting point to be 165°.

Hyoscyamine Picrate.—The residue from the tetrahydrofuran azeotrope extraction was treated with three small portions of water and centrifuged; the aqueous solutions were combined and treated with a saturated solution of picric acid. Crystallization was allowed to continue overnight. Recrystallization was effected from an alcohol-water solution. The melting point of the crystals obtained was found to be 165–166°. A mixed melting point of 165–166° was obtained with known hyoscyamine picrate. Henry (5) gives 165–166° for the melting point of hyoscyamine picrate.

Detection of Malic Acid.—In this portion of the investigation, approximately 1 Kg. of belladonna root was extracted by tetrahydrofuran azeotrope by Soxhlet extraction, using the appropriate size extractor. The extractive matter, after removal of the solvent, was treated with 100 cc. of water and gently heated to obtain solution of the water-soluble constituents and break up the mass. This mixture was then centrifuged, the aqueous portion was removed, and centrifuging was repeated twice.

The aqueous portions were combined, extracted with ether, evaporated to approximately 200 cc., and made slightly ammoniacal. To the ammoniacal solution was added 40% normal lead acetate, carefully avoiding an excess, until no further precipitation was noted.

The precipitated salts were carefully centrifuged with three 200-cc. portions of water. Final washing was with two 30-cc. portions of ethanol. The lead salts were suspended in water, decomposed with hydrogen sulfide, filtered, and decolorized with charcoal.

The method of Rosenthaler (6) was followed for the separation of the acids, and by his procedure only malic acid appeared to be present.

Free malic acid was obtained by an ether extraction (after reduction to about 30 cc.) using a Palkin extractor. The ether was evaporated and the residue was dissolved in 20 cc. of water. To this solution was added 4 Gm. of brucine alkaloid dissolved in 20 cc. of methanol. The methanol was evaporated by gentle heat, and crystallization of the brucine salt did not occur until the excess free brucine had been removed by extraction with chloroform.

Crystallization occurred overnight. The crystals were obtained by filtration and were washed with small portions of chloroform, acetone, and finally ether. The washed crystals were dried *in vacuo* over magnesium perchlorate. The melting point was determined to be 220–221° with decomposition. Recrystallization failed to raise the melting point.

Brucine *l*-malate was prepared in the same manner from known brucine and *l*-malic acid. The melting point of this compound was found to be 220–221° with decomposition. Mixed melting points were found to be 220–221°.

The specific rotation for both the compound in question and known brucine *l*-malate was determined in a 50–50 solution of methanol and chloroform as $[\alpha]_D^{25}$ −7.50 and $[\alpha]_D^{25}$ −7.62, respectively.

The crystals were studied under the microscope and appeared to be identical. Nieuwenburg and Brobbel (7) state that brucine *l*-malate can be identified in this manner.

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Acute and Short-Term Oral Toxicity Tests of Ferric Dimethyldithiocarbamate (Ferbam) and Zinc Dimethyldithiocarbamate (Ziram)*

By HAROLD C. HODGE, ELLIOTT A. MAYNARD, WILLIAM DOWNS, HARVEY J. BLANCHET, JR., and CHESTER K. JONES†

The increasing use of the fungicides ferbam and ziram makes imperative the presentation of additional information on their toxicities. When given in single doses intraperitoneally or orally, neither compound is highly toxic. Ferbam has a much lower acute toxicity than ziram. In thirty-day feeding tests, weanling albino rats grew normally when diets contained 0.01 per cent of either compound. Mortality was marked in groups fed an 0.5 per cent diet of ferbam. Tissues taken at the end of the tests of both compounds were normal histologically. Dogs tolerated 25 mg./Kg./day for one month without injury.

THE FERRIC and zinc derivatives of dimethyldithiocarbamic acid, known as ferbam and ziram, respectively, have attained an important status as agricultural fungicides. The wide usage of these materials has made it desirable to expand the limited knowledge of their toxicity (1, 2).

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EXPERIMENTAL

Acute Toxicity.—Ferbam and ziram have been administered intraperitoneally as aqueous suspensions to mice, rats, guinea pigs, and rabbits. Ferbam was given either as a 10% or 20% suspension; ziram was given either as a 2% or 5% suspension. Because the physical characteristics of the aqueous suspensions of the pure dithiocarbamates made injection by syringe difficult, most of the intraperitoneal injections were made with aqueous suspensions of the commercial wettable powders that contain 24% of inert ingredients (mostly clay), 0.2 to 0.5% of a surface active agent, either sodium lauryl sulfate or methyl naphthalene sulfate, neither of which was believed to have contributed significantly to the observed mortality. As shown in

Table I, ferbam given as a single dose intraperitoneally is practically nontoxic[1] in mice, rats, and guinea pigs, and is slightly toxic in rabbits. Ziram is slightly toxic in mice, rats, and guinea pigs and is moderately toxic in rabbits. No reactions on the part of the animals were observed in the first hour. Later the animals became stuporous and died in coma; survivors appeared to be entirely normal in twenty-four to forty-eight hours. Adhesions observed on gross autopsy after a two weeks' observation period were attributed to an irritant effect.

Aqueous suspensions of the concentrations just cited were given by stomach tube to rats, guinea pigs, and rabbits. Based on the results of these tests (Table I), ferbam is slightly toxic in guinea pigs and rabbits, and practically nontoxic in rats. Ziram is moderately toxic in guinea pigs and rabbits and slightly toxic in rats.

The acute toxicity of ferbam is much less than that of ziram. Given intraperitoneally, ferbam is $^1/_{10}$ to $^1/_{50}$ as toxic as ziram; given orally, ferbam is $^1/_7$ to $^1/_9$ as toxic as ziram. These compounds are characterized by flat dosage-response curves; the lethal doses can therefore be stated with less accuracy than is often achieved.

Short-Term Feeding Studies.—In preliminary tests, groups of 5 male albino rats of the Rochester strain (ex-Wistar) were given, at weaning, diets containing 0, 0.005, 0.05, 0.1, 0.3, and 0.5% of ferbam and of ziram, respectively, mixed in the diet of Purina Fox Chow Meal with meat. Additional groups were given diets containing 2% and 10% of ferbam. The two compounds had comparable effects on growth and mortality. Neither was highly toxic; the highest percentage that did not retard growth was 0.3% of ferbam and 0.1% of ziram. Up to 0.1% of either was tolerated without mortality.

Short-term feeding tests were carried out in which groups of 10 male and 10 female weanling albino rats were fed diets like those in the preliminary tests but with percentages as specified below. Food and tap water were supplied *ad lib.*

In the studies of ferbam, the rats were maintained for one month on diets containing 0 (control groups), 0.01, 0.05, 0.25, and 0.5% ferbam. The growth curves given in Fig. 1 (*A* and *B*) are averages of all rats in each group. The expected marked reduction in growth on the 0.5% diet did occur. Some growth was observed in rats given the 0.25% diet. Unexpectedly, particularly in the male rats, the groups given the 0.05% diet failed to gain as rapidly as the control rats. No reason for this difference from the results of the preliminary test is known. Additional groups of rats were given a diet containing 0.01% ferbam; the growth curves are practically identical with those of the control groups (Fig. 1, *B*). Only two rats of the 100 given diets containing 0.25% or less died. On the 0.5% diet, 50% of both male and female groups died; nearly all the deaths occurred during the third and fourth weeks. Blood samples were taken at the termination of the experimental period from the tail vein from 5 male and 5 female rats of the 0.25% and 0.5% groups, respectively. The hematological observations included red blood cell counts, hemoglobin values, white blood cell counts, and the incidence of a number of special forms of red blood cells. There was a slight and ill-defined tendency toward an anemia; otherwise the data fell within normal limits. At autopsy, organ weights were recorded for liver, kidneys, lungs, heart, brain, spleen, stomach, and testes; no abnormalities were observed in average organ weights. From rats at all dietary levels, sections of the following tissues were taken for histological examination: heart, lungs, spleen, stomach, small and large intestine, liver, kidneys, adrenal, testes, bone marrow, and brain. Samples of thymus and pancreas were occasionally included as well as samples of any abnormal tissue. The tissue sections were stained, using the conventional hematoxylin and eosin stains. There was no evidence of a regularly appearing tissue injury. Minor abnormalities such as pulmonary disease, pyelitis, a small granuloma in one liver, a slight fibrosis of the bone marrow, and minute focal areas of acute hepatitis were seen in at least one rat.

In the feeding studies of ziram, rats were maintained for one month on diets containing 0 (control groups), 0.01, 0.05, 0.25, and 0.5% ziram. The growth curves are shown in Fig. 1 (*C* and *D*). The growth retardation was marked in the 0.5% groups, and somewhat less, though still well established, in the 0.25% groups. When the 0.05% groups unexpectedly showed a slight but possibly significant retardation, additional groups were tested, using diets containing 0.01% ziram. The growth equaled or surpassed (Fig. 1, *D*) that of the control groups.

[1] For definitions of the toxicity classes, see Hodge, H. C., and Sterner, J. H., *Am. Ind. Hyg. Assoc. Quart.*, **10**, 4(1949).

TABLE I.—ACUTE TOXICITY DATA[a]

		Ferbam				Ziram			
		Sex	No.	LD_{50}[b]	Lethal Range[c]	Sex	No.	LD_{50}[b]	Lethal Range[c]
Intraperitoneal	Mice	M	80	3000 ± 230		F	62	73 ± 11	
	Rats	M	80	2700 ± 96		M	94	23 ± 2	
						F	60	33 ± 5	
	Guinea Pigs	M, F	9		700–2300	M, F	6		20–30
	Rabbits	M	11		200–1500	M	12		5–50
Oral	Rats	F	30		17000[d]	F	96	1400 ± 99	
	Guinea Pigs	M	6		450–2000	M	8		100–150
	Rabbits	M	8		2000–3000	M	12		100–1020

[a] Values are given in mg./Kg. of body weight.
[b] LD_{50} ± standard error.
[c] The lower figure represents the upper limit for survival of all animals tested; the higher figure represents the lower limit for doses that proved fatal in all animals tested.
[d] Killed only one of ten.

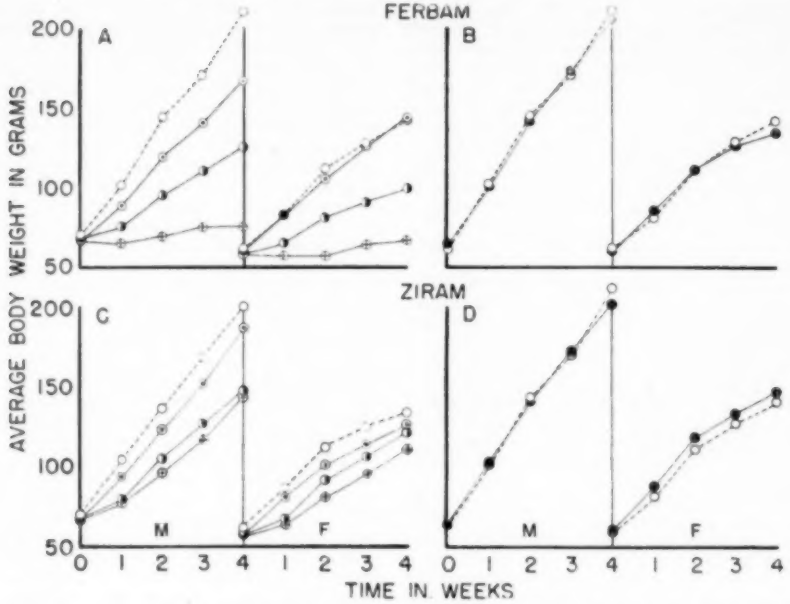

Fig. 1.—Growth curves of male (M) and female (F) weanling albino rats (10 rats per group) during four-week experimental periods. Upper curves, ferbam; lower curves, ziram. In parts A and C, the diets contained the following percentages: ○—controls, ◓—0.05%, ◑—0.25%, ⊕—0.5%. In parts B and D, the diets contained as follows: ○—controls, ●—0.01%.

Of the 120 rats, only 3 died; one female in the 0.25% group and one male and one female in the 0.5% groups. Blood samples were taken near the end of the experimental period from 5 male and 5 female rats in each group given the 0.5% and 0.25% diets. Except for a vague tendency toward anemia, the values lay in normal ranges. No evidence of any abnormality was seen in the average organ weights. No histological lesions were discovered that were attributed to the inclusion of ziram in the diet. Pathology of minor degree, e. g., pulmonary disease, low-grade kidney involvement, an intestinal parasite, an instance of acute pericarditis, slight liver changes, and scattered eosinophils in the submucosa of the stomach, were seen in control as well as in experimental rats.

One dog was given 50 mg./Kg./day of ferbam, and another dog was given the same dose of ziram by capsule for one week; an attempt to double this dose immediately provoked severe vomiting and malaise. After a recovery period, the dogs consumed 25 mg./Kg./day without illness for one month. An additional dog was given 5 mg./Kg./day of each compound, respectively, for one month with no sign of any toxic effect. Body weight was maintained. Normal urinary protein and sugar values were found terminally. Blood samples taken prior to starting the experiment and again near termination gave normal values, although there was a decrease in red blood cell counts and hemoglobin values in the dogs given ferbam. Organ weights were normal. Thorough histological study gave no evidence of tissue damage that could be attributed to the treatment.

DISCUSSION

The mechanism of action of the ferric and zinc dimethyldithiocarbamates is not understood. There are frequent suggestions that these and related compounds produce, as a metabolic product, carbon disulfide which is responsible for part or all of the toxic effects. Although no work was done on the mechanism of action, the lack of goitrogenic activity has been demonstrated. Seifter and Ehrich (3) screened 78 compounds for goitrogenic action. Of the 4 found to be markedly active, one, disodium ethylene bisdithiocarbamate (Nabam), is a not-distant chemical relative of the two salts currently under study. To rule out the possibility of thyroid effects from the administration of the ferric and zinc salts, groups of 10 male and 10 female rats were maintained for one month on diets containing 0 (control groups) and 0.25% of ferbam and ziram, respectively. The rats were sacrificed and the thyroid glands were prepared (using hematoxylin and eosin stains) for microscopic examination. In every case only normal thyroid tissue was found, thereby establishing a complete absence of any goitrogenic effect. The thyroids of the dogs were normal also. The dog, however, has a resistant thyroid; the rat test is a more delicate and reliable indication of the absence of goitrogenic action.

Additional data on the chronic toxicity of ferbam and ziram are currently being obtained. Feeding studies have been in progress for nearly a year in which the following percentages of each compound are mixed into the diets of albino rats: 0, 0.0025, 0.025, and 0.25%. The groups of rats, both males and females, on the 0.25% rations are exhibiting a diminution in growth and somewhat greater mortality than the control groups. Hemograms are normal. Dogs in pairs have been fed 0.5, 5, and 25 mg./Kg./day of ferbam and ziram, respectively, for approximately six months with no consistent evidence of injury.

SUMMARY

1. The acute toxicity of ferbam is low. A single dose of ferbam administered intraperitoneally is practically nontoxic in guinea pigs, rats, and mice (lethal dose, 1,000–3,000 mg./Kg.) and only slightly toxic in rabbits (200–700 mg./Kg.). Given by stomach tube, ferbam is slightly toxic in guinea pigs and rabbits (700–3,000 mg./Kg.) and practically nontoxic in rats (17,000 mg./Kg. killed 1 of 10).

2. Administered intraperitoneally, ziram is moderately toxic in rats, mice, guinea pigs, and rabbits (5–73 mg./Kg.). Given by stomach tube, ziram is moderately toxic in guinea pigs and rabbits (100–300 mg./Kg.) and slightly toxic in rats (1,400 mg./Kg.).

3. In short-term feeding tests of ferbam, groups of ten male and ten female weanling albino rats were fed diets containing 0, 0.01, 0.05, 0.25, and 0.5 per cent for one month. No growth retardation was noted in the 0.01 per cent groups; higher percentages progressively depressed growth. Mortality was marked only in the 0.5 per cent groups. Average organ weights were normal. There were no histological changes.

4. In short-term feeding tests with ziram, comparable groups of male and female rats were fed diets containing the percentages listed for ferbam. Growth was normal in the 0.01 per cent groups but higher percentages progressively depressed growth. Mortality was not marked even in the 0.5 per cent groups. Organ weights were normal.

5. In an additional test, only normal thyroid tissue was found in rats that had ingested diets containing 0.25 per cent ferbam and ziram, respectively, for one month.

6. Dogs given 5 and 25 mg./Kg./day, respectively, either of ferbam or of ziram, survived for one month without illness. Hematological values, urine analyses, and organ weights were normal. Histological studies did not show tissue damage.

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1953 Iodine Research Award Nominations Requested

Nominations are now being received by the AMERICAN PHARMACEUTICAL ASSOCIATION for the 1953 Chilean Iodine Educational Bureau Award recognizing outstanding research in the chemistry and pharmacy of iodine and its compounds as applied in pharmacy or medicine. Any member of the ASSOCIATION may propose a nominee by submitting eight copies of each of the publications to be considered in the competition, a biographical sketch of the nominee, including date of birth, and a list of his publications to Robert P. Fischelis, Secretary of the AMERICAN PHARAMACEUTICAL ASSOCIATION, 2215 Constitution Ave., N. W. Washington 7, D. C. Nominations must be received on or before February 1, 1953.

A nominee must be a resident of the United States or Canada. During the period covered by the nomination, he shall have been actively engaged in, have completed, or have published a report upon the line of investigation for which the award is made. During the period of two years prior to the date of nomination, he shall not have been engaged in research under the sponsorship of the Chilean Iodine Educational Bureau, Inc.

The award, consisting of $1,000 and a diploma setting forth the reasons for selection of the recipient, will be presented at the annual meeting of the ASSOCIATION. At this meeting, the recipient will deliver a paper or lecture upon the subject of his scientific work which will then be published in the JOURNAL OF THE AMERICAN PHARAMACEUTICAL ASSOCIATION. He will receive an allowance of not more than $250 to defray his expenses in attending the meeting.

The recipient will be selected by an award committee appointed by the chairman of the ASSOCIATION's Council. It includes Justin L. Powers, chairman; Louis Gershenfeld, Charles O. Wilson, Harvey B. Haag, Heber W. Youngken, John C. Krantz, and Frank O. Taylor.

Studies on Species of *Asclepias.* VII. Determination of Palisade Ratio*,†

By W. E. HASSAN, Jr., R. A. GOSSELIN, R. W. VANDER WYK, and M. W. QUIMBY

The determination of palisade ratios for six species of the genus *Asclepias* is presented. After these ratios are obtained, their reliability as diagnostic aids in the differentiation of closely related species is determined by the application of the "Statistical Quality Control Chart Technique" of industry. By the use of this method, it is possible to distinguish one species from another.

WITHIN the past decade, many investigators in the field of pharmacognosy have demonstrated the value of the palisade ratio in the identification of closely related species of plants (1–4).

In an earlier paper dealing with the comparative anatomy of the leaves of six species of *Asclepias* (5), it was stated that palisade ratio studies were being undertaken. The present report gives the results of that investigation, in which palisade ratios of the 6 species of *Asclepias* were determined and treated statistically to ascertain whether the measures obtained can be of diagnostic value in the identification of medicinally employed *A. tuberosa* L. from the other species studied.

METHODS AND PROCEDURE

The method of preparing the leaf samples and the procedure employed for the determination of palisade ratio are the same as those described in a previous paper (6). Table I shows the results obtained for the apex, center, margin, base, and mean palisade ratio for *Asclepias tuberosa* L., *Asclepias syriaca* L., *A. incarnata* L. var. *pulchra* Hort., *A. incarnata* L. var. *alba* Hort., *A. curassavica* L., and *A. speciosa* Torr.

In order to determine the statistical reliability of the mean palisade ratios obtained in this investigation, a graphic method (Fig. 1) involving the use of statistical control charts was employed. As the first step in employing the control chart as a test of significance for the 6 mean palisade ratios, it was assumed that there was no real difference between these several ratio measures, or, in other words, that the actual numerical differences between them were due only to chance errors inherent in the counting technique. If the assumption of no real difference can be accepted, the mean palisade ratios determined for the 6 species are of no value as diagnostic aids. If the assumption is rejected, then it is apparent that the differences are real or significant, due to the fact that ratios were determined for different species of *Asclepias*.

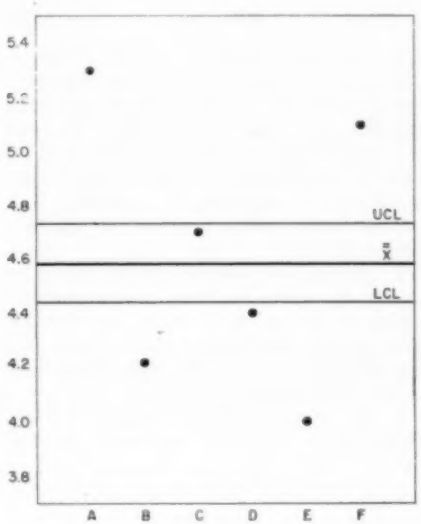

Fig. 1.—Control chart for the palisade ratios of six species of the genus *Asclepias*.

The data used in constructing the control chart (Fig. 1) were obtained as follows:

1. The arithmetic mean ($\bar{X}$) for 100 separate palisade ratio counts for each species is determined.
2. A grand mean ($\bar{\bar{X}}$) for all six species is computed.
3. The range (R) of each series of ratios is determined by subtracting the lowest ratio values from the highest ratio value of the species.
4. The average range ($\bar{R}$) is computed for all 6 species.
5. The standard error of the mean is obtained by making use of a table of factors which enables this statistic to be determined from average range.
6. Upper and lower control limits, representing the range within which it is expected that mean palisade ratios will vary due to chance errors alone, are computed.
7. The grand mean and the upper and lower control limits are indicated on an arithmetic grid.

* Received August 22, 1952, from the Massachusetts College of Pharmacy, Boston.
Presented to the Scientific Section, A. PH. A., Philadelphia meeting, August, 1952.

† Abstracted from a thesis submitted to the Graduate Council of the Massachusetts College of Pharmacy by W. E. Hassan in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

TABLE I.—MEAN PALISADE RATIOS FOR SIX SPECIES OF ASCLEPIAS

Species	Location on Leaves				Arithmetic Mean
	Apex, Av.	Center, Av.	Margin, Av.	Base, Av.	
A. tuberosa	5.7	5.1	5.8	4.5	5.3
A. syriaca	5.0	4.5	3.7	3.4	4.2
A. incarnata var. *pulchra*	4.7	5.1	4.8	4.0	4.7
A. incarnata var. *alba*	4.5	3.8	4.7	4.4	4.4
A. curassavica	4.5	4.0	3.9	4.0	4.0
A. speciosa	5.3	5.1	5.0	4.9	5.1

The individual mean palisade ratios for the six species are plotted and the resulting chart is analyzed for evidence of lack of statistical control.

The computations required in this investigation are presented in Table II.

CALCULATION OF DATA

Values used in the calculation of data were as follows: grand mean ($\bar{\bar{X}}$), 4.6; average range ($\bar{R}$), 2.5; number of observations per species (n), 100; factor d_2 for estimating universe standard deviation (S. D.) from average range of sample size of 100, 5.015.[1]

The following formulas were employed:

$$\text{Standard Deviation (S. D.)} = \frac{\bar{R}}{d_2} = \frac{2.5}{5.015} = 0.498 = 0.5$$

$$\text{Standard Error of Mean (S. E.}_{\bar{X}}) = \frac{\text{S. D.}}{\sqrt{n}} = \frac{0.5}{\sqrt{100}} = 0.05$$

$$\text{Upper Control Limit (U. C. L.)} = \bar{\bar{X}} + 3(\text{S. E.}_{\bar{X}}) = 4.6 + 3(0.05) = 4.75$$

$$\text{Lower Control Limit (L. C. L.)} = \bar{\bar{X}} - 3(\text{S. E.}_{\bar{X}}) = 4.6 + 3(0.05) = 4.45$$

INTERPRETATION AND CONCLUSIONS

The control chart (Fig. 1) clearly indicates a lack of statistical control. Five of the 6 mean ratios are beyond the limits of chance variation. In a pure chance system, 99.73 per cent of the cases lie between the range mean $\pm 3\sigma_{\bar{X}}$, represented in this case by the upper and lower control limits. The original assumption of no real difference is, therefore, rejected. The control chart indicates that *A. tuberosa* can be differentiated from *A. syriaca*, *A. incarnata* var. *alba*, *A. incarnata* var. *pulchra*, and *A. curassavica*. While there is statistical evidence that *A. speciosa* can also be differentiated from *A. tuberosa* by comparing their respective mean palisade ratios, the close proximity of these 2 measures presents a doubtful significance and is therefore not considered as being of practical value as a diagnostic aid.

[1] Table B, Factors for estimating σ' from $\bar{R}$ or $\bar{s}$, E. L. Grant, "Statistical Quality Control," McGraw-Hill Book Co., 1946.

TABLE II.—COMPUTATIONS USED

Species	Mean Palisade Ratio, $\bar{X}$	Individual Palisade Ratios		Range (R)
		Low	High	
A. tuberosa	5.3	4.0	6.5	2.5
A. syriaca	4.2	3.0	6.3	3.3
A. incarnata var. *pulchra*	4.7	3.0	6.0	3.0
A. incarnata var. *alba*	4.4	3.5	5.4	1.9
A. curassavica	4.0	3.0	5.3	2.3
A. speciosa	5.1	4.0	6.0	2.0
$\bar{\bar{X}}$ =	4.6		$\bar{R}$ =	2.5

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Studies on Species of *Asclepias*. VIII. Determination of Vein Islet Numbers*,†

By W. E. HASSAN, Jr., R. A. GOSSELIN, R. W. VANDER WYK, and M. W. QUIMBY

The determination of vein islet numbers for 6 species of the genus *Asclepias* is described. After these numbers are obtained, their reliability as diagnostic aids in the differentiation of closely related species is determined by the application of the "Statistical Quality Control Chart Technique" of industry. By the use of this method, it is possible to distinguish one species from another.

DURING the past few years, many investigators have published the results of vein islet number determinations upon various species of medicinal plants. In each instance the statistically treated results have proved to be of diagnostic value in the identification of closely related plants. The purposes of this investigation are to present the results of the vein islet number determinations performed upon *Asclepias tuberosa* L., *Asclepias syriaca* L., *Asclepias speciosa* Torr., *Asclepias curassavica* L., *Asclepias incarnata* L. var. *pulchra* Hort., and *Asclepias incarnata* L. var. *alba* Hort., and to utilize these numbers in the construction of a control chart in order to determine the reliability of the vein islet number as a diagnostic aid in pharmacognosy.

HISTORY

Eames and MacDaniels (1) define a vein islet as " . . . the ultimate divisions of the conducting strands completely or partially encircling minute areas of photosynthetic tissues with which they are in close contact." Benedict (2) has given the name "vein islet" to the small area of the leaf enclosed by the smallest branching veins.

The term "vein islet number" was coined by Levin (3) who defined it as "the number of vein islets in a square millimeter of leaf tissue." The vein islet numbers referred to in this investigation correspond to those which were defined by Levin.

METHOD

In the examination of this plant material, a method proposed by Zufall and Burlage (4) was employed.

The technique of calculation employed for the construction of the control chart (Fig. 1) is fully described in an accompanying paper dealing with the palisade ratio in the leaves of these same species of *Asclepias* (5).

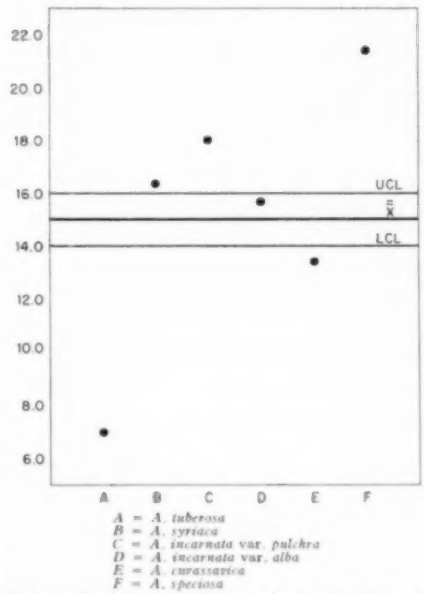

Fig. 1.—Control chart for the vein islet numbers of six species of the genus *Asclepias*.

DISCUSSION

Table I was constructed to show the lowest recorded V. I. N., the highest recorded V. I. N., the range, the median, mode, and arithmetic mean of the six species studied. The range and arithmetic mean are required for the construction of the control chart. The median and mode are indications of the nature of the distribution of vein islet numbers and of the representativeness of the arithmetic mean.

There is good evidence, even before statistical treatment, that the mean vein islet number for each species can be used as a diagnostic aid, in that each value is markedly different from the other. *A. tuberosa* yields the lowest mean value, while *A. speciosa* yields the highest. *A. tuberosa*, the most widely used medicinal member of the genus, can be differentiated from all other members by making use of the vein islet number technique.

Table II is presented solely to show the distribution of vein islets on the major positions of the leaves, the apex, center, margin, and base.

An inspection of Fig. 1 (control chart for vein islet number) indicates that all the sample averages are

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Presented to the Scientific Section, A. Ph. A., Philadelphia Meeting, August, 1952.
† Abstracted from a thesis submitted to the Graduate Council of the Massachusetts College of Pharmacy by W. E. Hassan, Jr., in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

TABLE I.—VEIN ISLET NUMBERS

Species	Lowest Recorded V. I. N.[a]	Highest Recorded V. I. N.[a]	Range	Median	Mode	Arithmetic Mean
A. tuberosa	5.0	12.0	7.0	7.0	7.0	7.1
A. speciosa	16.5	32.0	15.5	21.0	18.5	21.4
A. syriaca	11.0	34.0	23.0	15.0	14.0	16.4
A. curassavica	11.0	16.0	5.0	13.0	14.0	13.2
A. incarnata var. *pulchra*	13.5	25.0	11.5	18.0	16.5	18.2
A. incarnata var. *alba*	10.0	27.5	17.5	16.0	16.0	15.6

[a] V. I. N. = Vein Islet Number.

TABLE II.—DISTRIBUTION OF VEIN ISLETS

Species	Position			
	Apex	Center	Margin	Base
A. tuberosa	8.1	7.4	7.0	5.7
A. speciosa	24.1	20.1	22.7	18.6
A. syriaca	21.1	15.1	15.5	14.0
A. curassavica	13.7	13.2	12.9	13.0
A. incarnata var. *pulchra*	20.6	16.4	19.6	16.2
A. incarnata var. *alba*	15.8	16.7	13.6	16.2

significantly different from one another. Since this is so, it is possible to state that, by using the figures obtained by a vein islet number determination, one is able to differentiate among these 6 species of the genus *Asclepias*.

SUMMARY AND CONCLUSIONS

1. The vein islet numbers for the above-mentioned members of the genus *Asclepias* have been presented for the first time.

2. The mean vein islet number for each species is as follows: *A. tuberosa*, 7.1; *A. speciosa*, 21.4; *A. syriaca*, 16.4; *A. curassavica*, 13.2; *A. incarnata* var. *pulchra*, 18.2; and *A. incarnata* var. *alba*, 15.6.

3. A table showing the distribution of vein islets on the leaf areas is presented.

4. The control chart technique has been applied to the vein islet number determination to prove the reliability of the vein islet number as a diagnostic aid.

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CORRECTION

In the article entitled, "Biosynthesis and Metabolism of Some Radioactive *Veratrum viride* Alkaloids," by A. Pircio and E. M. K. Geiling, [THIS JOURNAL, **41**, 552–554 (October, 1952)] the following errors were made in stating the physical properties of the alkaloids: Page 553, left-hand column, line 15, etc., should read, "Germidine: m. p., 229–231°; $[\alpha]_D^{25} = +12°$ (in chloroform); specific activity = 23,050 C. P. M./mg." Following this should be inserted: "Veratrosine: m. p., sintered at 235° and melted between 243–245°; $[\alpha]_D^{25} = -55°$ (in equal volumes of ethanol and chloroform); specific activity = 27,700 C. P. M./mg.

The Spectrophotometric and Polarographic Determination of Isonicotinic Acid Hydrazide*

By J. D. NEUSS, W. J. SEAGERS, and W. J. MADER†, ‡

Instrumental methods are described for the identification and assay of isonicotinic acid hydrazide in the crystalline form and in tablets. Infrared absorption spectrophotometry offers a highly specific means for qualitative differentiation from isomers and closely related compounds. Ultraviolet absorption spectrophotometry provides a simple means of assay, which, while not specific in the presence of related compounds, does differentiate from isomers by means of the wave lengths and absorbancy ratio at the maximum and minimum. Polarography affords an alternate assay method free from interference by isomers and related compounds.

SINCE the announcement early this year (1–3) of the antitubercular activity of isonicotinic acid hydrazide (isoniazid) and certain of its derivatives, there has been considerable interest in this class of compounds. While final judgment has not been passed on their efficacy, particularly as to the development of resistant strains of tubercle bacilli, considerable quantities of isonicotinic acid hydrazide have been produced in this country. Obviously, suitable methods of identification and assay are desirable in the quality control of the substance and its formulations.

The structural formula of isonicotinic acid hydrazide is shown in Fig. 1. The strong reducing action of the hydrazine moiety is suggestive of methods based on oxidimetry, and a method has been published (4) involving titration with iodine. Our studies were concerned with the development of more specific methods of assay and identification. Infrared absorption spectrophotometry was found to be highly specific in the identification and differentiation from the isomers, nicotinic acid hydrazide and picolinic acid hydrazide, as well as the corresponding acids. Ultraviolet absorption spectrophotometry provides a rapid method for the assay of commercial lots of the crystalline substance and tablet formulations. Polarography offers an even more specific method of assay, which suffers no interference from the isomers or related compounds.

INFRARED ABSORPTION SPECTROPHOTOMETRY

The samples were prepared for infrared examination by grinding about 20 mg. with a mortar and pestle, followed by mulling with about 0.05 ml. of liquid petrolatum. The infrared spectra were recorded on a single-beam, model IR-2 Beckman spectrophotometer, using a sodium chloride prism and a sodium chloride cell without spacers. The wave-length scale was calibrated against the atmospheric carbon dioxide band at 4.22 μ and the benzene maximum at 9.64 μ.

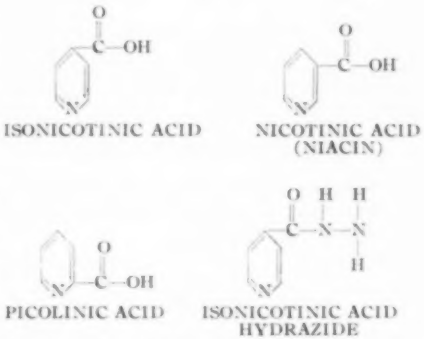

Fig. 1.—Structural formulas of isonicotinic acid hydrazide and related compounds.

The spectrum of isonicotinic acid hydrazide is shown in Fig. 2 together with that of isonicotinic acid. The chief features are a strong carbonyl band at 6.00 μ (not shown in Fig. 2) and bands at 8.20, 8.78, 9.48, 10.05, 11.25, and 11.82 μ. A weak band, sometimes absent, may appear at 9.71 μ. These maxima are reproducible to within ± 0.02 μ. The strong bands at 10.05 and 11.82 μ serve to distinguish isonicotinic acid hydrazide from its isomers and certain other related compounds. Neither nicotinic acid hydrazide nor picolinic acid hydrazide exhibits bands at 10.05 or 11.82 μ. Instead, the former shows a shift of the 11.82-μ band to 11.98 μ, and the latter a shift to 12.19 μ. This displacement effect is similar to that shown by other 2-, 3-, and 4-monosubstituted pyridine isomers or disubstituted benzene isomers (5).

Identification of isonicotinic acid hydrazide in tablets requires preliminary separation by extraction in order to avoid interference by lactose, starch, or other excipients. A sample of powdered tablets, equivalent to about 50 mg. of isonicotinic acid hy-

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Presented to the Scientific Section, A. Ph. A., Philadelphia meeting, August, 1952.
† The authors gratefully acknowledge the aid of August Doering who performed the ultraviolet measurements and Edward Schulz who performed the infrared measurements.
‡ After this paper had been accepted for publication, a paper by P. G. W. Scott [*J. Pharm. Pharmacol.*, **4**, 681(1952)] described polarographic and U. V. spectrophotometric methods substantially the same as those described herein.

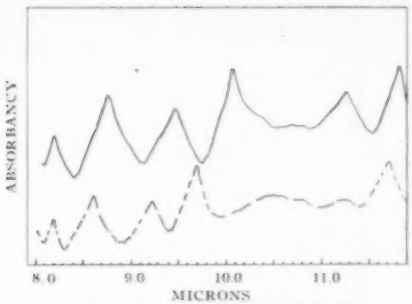

Fig. 2.—Infrared spectra. — Isonicotinic acid hydrazide; ---- isonicotinic acid. Liquid petrolatum mulls.

drazide, is shaken with 25 ml. of chloroform. After filtration, the clear filtrate is evaporated to dryness on the steam bath. About 20 mg. of the dry residue is thoroughly mulled with about 0.05 ml. of liquid petrolatum. The infrared spectrum is recorded from 5.8 to 6.2 μ and from 7.9 to 12 μ. The seven characteristic absorption bands mentioned above should be found.

ULTRAVIOLET ABSORPTION SPECTROPHOTOMETRY

The ultraviolet absorption spectrum of isonicotinic acid hydrazide in water solution exhibits a maximum at 262 mμ and a minimum at about 227 mμ, as shown in Fig. 3. In dilute aqueous hydrochloric acid, the maximum is shifted to 267 mμ and the minimum to 235 mμ. In dilute aqueous sodium hydroxide, a maximum occurs at about 300 mμ and a minimum at about 250 mμ; however, the alkaline solution is unstable and the spectrum changes rapidly. Comparison of the chief ultraviolet absorption characteristics of isonicotinic acid hydrazide with its isomers and with isonicotinic acid and nicotinic acid is given in Table I. These data indicate that the compounds listed can be qualitatively differentiated by ultraviolet absorption. Quantitative assay of samples of crystalline isonicotinic acid hydrazide or tablets were performed by the following procedures. Because the spectrum exhibits a sharper maximum in dilute acid than in water, 0.1 *N* hydrochloric acid was selected as the solvent for assay purposes.

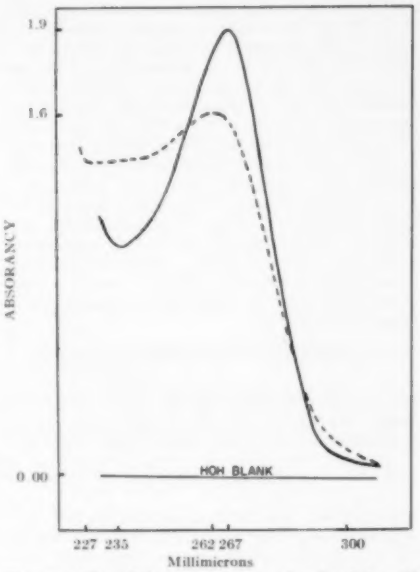

Fig. 3.—Ultraviolet spectra. — Isonicotinic acid hydrazide in 0.1 *N* HCl; ---- isonicotinic acid hydrazide in water.

U. V. Assay of Crystalline Isonicotinic Acid Hydrazide.—Weigh accurately about 75 mg. of sample, dilute to 500 ml. with water, and shake until dissolved. Transfer 10.00 ml. by pipette to a 100-ml. volumetric flask, add 10.0 ml. of 1.00 *N* hydrochloric acid, dilute with water to the mark, and mix. Measure the absorbancies in a 1-cm. quartz cell in the vicinity of 267 and 235 mμ with a Beckman DU spectrophotometer, using 0.1 *N* hydrochloric acid (prepared by dilution of the 1.00 *N* hydrochloric acid) as the reference liquid. An absorption maximum should be found at 267 ± 1 mμ and a minimum at 235 ± 2 mμ. Calculate the assay from the following expression:

Percent isonicotinic acid hydrazide = $13370\ A/S$

where A is the absorbancy at 267 mμ and S is the weight of sample in milligrams.

U. V. Assay of Tablets.—Weigh accurately a counted number of not less than 20 tablets and re-

TABLE I.—U. V. ABSORPTION CHARACTERISTICS OF ISONICOTINIC ACID HYDRAZIDE, ITS ISOMERS, AND RELATED ACIDS

Substance	Solvent	λ max., mμ	A 1%, 1cm. max.	λ min., mμ	A 1%, 1cm. min.	A max. / A min.
Isonicotinic acid hydrazide	water	262	304	227	254	1.20
	0.1 *N* HCl	267	374	235	193	1.94
Nicotinic acid hydrazide	water	261	302	247	275	1.10
	0.1 *N* HCl	261	396	238	157	2.52
Picolinic acid hydrazide	water	265	398	244	274	1.45
	0.1 *N* HCl	266	438	244	170	2.58
Isonicotinic acid	water	262	333	233	192	1.73
	0.1 *N* HCl	270	355	238	72	4.93
Nicotinic acid	water	261	360	237	123	2.93
	0.1 *N* HCl	261	424	233	52	8.15

duce them to a fine powder without appreciable loss. Weigh accurately a portion equivalent to about 75 mg. of isonicotinic acid hydrazide, dilute with water to 500 ml. in a volumetric flask, and shake well for several minutes. Filter through a fine-porosity sintered-glass filter, rejecting the first portion of the filtrate. Dilute 10.00 ml. of the clear filtrate plus 10.0 ml. of 1.00 *N* hydrochloric acid to 100 ml. with water in a volumetric flask, and mix. Measure the absorbancies in a 1-cm. quartz cell in the vicinity of 267 and 235 mμ with a Beckman DU spectrophotometer, using 0.1 *N* hydrochloric acid (prepared by dilution of the 1.00 *N* hydrochloric acid) as the reference liquid. An absorption maximum should be found at 267 ± 1 mμ and a minimum at 235 ± 2 mμ. Calculate the assay from the following expression:

$$\text{Mg. of isonicotinic acid hydrazide per tablet} = \frac{133.7\,(A)\,(T)}{W}$$

where A is absorbancy at 267 mμ, T is average weight per tablet, and W is sample weight.

The precision of these methods is about 1 to 2%.

POLAROGRAPHY

Isonicotinic acid hydrazide exhibits two well-defined polarographic waves (Fig. 4) in the *p*H range 1.5 to 7, which gradually merge into a single wave at a *p*H of 8 or higher. This behavior is shown in Fig. 5 where the half-wave potential is plotted *vs.* *p*H. It was found that both wave heights are directly proportional to concentration and hence suitable for assay purposes. Although isonicotinic acid, ethyl isonicotinate, nicotinic acid, nicotinic acid hydrazide, and picolinic acid hydrazide do not interfere in the *p*H range 1.5 to 5, a *p*H of 1.5 was selected as convenient for the assay of both crystalline isonicotinic acid hydrazide and tablets. At this *p*H, the half-wave potentials of the two waves are respectively −0.52 and −0.70 v. *vs.* S. C. E.

Procedure.—In order to determine the polarographic behavior of isonicotinic acid hydrazide, a 0.0005 *M* solution of purified material (99.3%) was prepared in a universal buffer which was 0.1 *M* in phosphoric, acetic, and boric acids and 0.5 *M* in potassium chloride. Various amounts of 1 *M* sodium hydroxide were added to aliquots of this solution in order to obtain the desired range of *p*H values. These were measured with a Beckman Model G *p*H meter calibrated at *p*H 4.01 and 6.86 with standard buffers. Polarograms were recorded with a Leeds & Northrup Type E Electrochemograph in conjunction with an H-type cell (6) maintained at 25° and a conventional dropping-mercury electrode. Oxygen was removed from the solution by bubbling for five minutes with nitrogen purified by passage through an alkaline solution of pyrogallol.

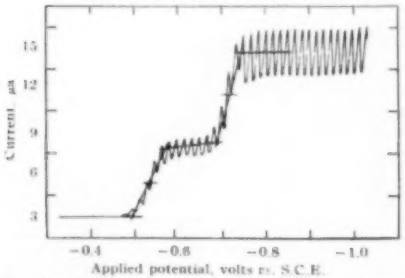

Fig. 4.—A typical polarogram of isonicotinic acid hydrazide at *p*H 1.5 in a universal buffer.

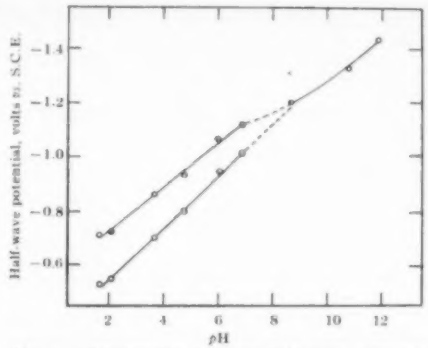

Fig. 5.—Relationship between half-wave potential and *p*H for isonicotinic acid hydrazide in a universal buffer.

In the assay of crystalline material, about 50 mg. was accurately weighed, transferred to a 250-ml. volumetric flask, and diluted to volume with water. For the assay of tablets, 5 tablets were triturated with water, transferred quantitatively to a 1-L. volumetric flask, and diluted to volume with water. Filtering was unnecessary since it was found that the corn starch, lactose, and magnesium stearate present in the tablets did not interfere with this assay. A standard solution was prepared by weighing accurately 50 mg. of isonicotinic acid hydrazide of known purity [99.3% as determined by phase solubility measurements (7)], transferring to a 250-ml. volumetric flask, and diluting to volume with water. The cell solution in all cases was prepared by mixing 10.0 ml. of sample solution or standard solution, respectively, with 10.0 ml. of an aqueous buffer solution which contained 14 ml. of 85% phosphoric acid, 12 ml. of glacial acetic acid, 12.4 Gm. of boric acid, and 74.6 Gm. of potassium chloride (all reagent grade chemicals) per liter of solution. The cell solution was deoxygenated for five minutes with purified nitrogen and polarographed between 0 and −1.0 volts *vs.* S. C. E. at a suitable sensitivity. Both wave heights were measured and calculated in microamperes. The appropriate values were substituted in the following formulas, using the wave heights for corresponding waves of standard and sample solutions. In order for the formulas to be valid, the height of the mercury column above the tip of the capillary must remain constant for both standard and sample. For crystalline isonicotinic acid hydrazide the formula used was:

$$\text{Per cent isonicotinic acid hydrazide} = \frac{\text{mg. std. used}}{\text{mg. sample}} \times \frac{\text{wave height, sample (μa)}}{\text{wave height, std. (μa)}} \times \%\ \text{purity of std.}$$

For tablets the formula used was:

$$\text{Mg. of isonicotinic acid hydrazide per tablet} = \text{mg. std. used} \times \frac{\text{wave height, sample } (\mu a)}{\text{wave height, std. } (\mu a)} \times \frac{4}{5} \times \frac{\% \text{ purity of std.}}{100}$$

The precision of these assays is ±1%.

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Composition of Gum Turpentines of Pines. XIV. A Report on Three Mexican Pines: *Pinus ayacahuite, Pinus cembroides,* and *Pinus pinceana**

By N. T. MIROV

The composition of gum turpentines from three species of Mexican pines has been determined. Each sample was fractionated and its components have been investigated and reported.

PINUS AYACAHUITE Ehrenb. is known in the United States as Mexican white pine. The tree looks like the western white pine, *Pinus monticola* Dougl., but its large cones with peculiar curly scales are different from the cones of *Pinus monticola*. Apparently there exists a gradual change in appearance of the cone from the typical *Pinus monticola* to the typical *Pinus ayacahuite* of Mexico and Guatemala. An intermediate form growing in the northern parts of Mexico and in the adjacent parts of the southwest United States is known as *Pinus reflexa* Engelm, which will be reported in the next part of this series.

EXPERIMENTAL

The oleoresin used in the present experiments was collected by the author with the kind assistance of Sr. Ing. Marcial Zebadua, forester for the State of Chiapas.[1]

Twenty carefully identified trees yielded about 2,000 Gm. of oleoresin, silky in appearance, noncrystalline, and rather dark in color because of rust and contamination.

The turpentine was distilled from the oleoresin *in vacuo*. At the beginning of the distillation, some volatile oil distilled at room temperature under 40 mm. pressure. At the end of the distillation, the pressure was 1 mm. and the temperature was 200°. The crude turpentine had the following characteristics: yield, 17% of the crude oleoresin; d_4^{25}, 0.8194; n_D^{24}, 1.4528; $[\alpha]_D^{25}$, +7.93°. It is seen from these data that the turpentine possessed rather lower density and index of refraction than most turpentines.

A batch of 300 Gm. of the turpentine was fractionated in a jacketed and heated column 80 cm. long, equipped with a stillhead permitting 1:10 distillation reflux ratio. The results of the fractional distillation are shown in Table I.

Combined fractions 1 and 2 were repeatedly shaken with fuming sulfuric acid and redistilled at atmospheric pressure. The physical constants of this purified material are given in Table II.

From this evidence it is concluded that fractions 1 and 2 and the heads of fraction 3 contained a considerable quantity of *n*-heptane (C_7H_{16}).

A portion of fraction 3 was redistilled over metallic sodium at atmospheric pressure. A small part distilled over from 100–155°. Most of the distillate, however, was collected at 155°. α-Pinene was identified in this fraction by preparing crystalline nitrosochloride which, after several precipitations from chloroform (with methanol), melted at 103°. Nitrosopiperidine, prepared from the nitrosochloride, melted at 118–119°.

Fraction 4, amounting to 12 Gm., was redistilled over metallic sodium at atmospheric pressure in a

* Received June 3, 1952, from the California Forest and Range Experiment Station, maintained by the Forest Service, U. S. Department of Agriculture, in cooperation with the University of California, Berkeley.

[1] The oleoresin collecting trip to Mexico was made by the author in the summer of 1950 with the assistance of the Associates of Subtropical Geography, University of California. Thanks are due to Dr. Carl O. Sauer of the Department of Geography of this University for his interest in the project.

TABLE I.—FRACTIONAL DISTILLATION OF TURPENTINE OF PINUS AYACAHUITE, P. CEMBROIDES, AND P. PINCEANA

Fractions	Pressure, Mm.	Boiling Range, °C.	Distillate, %	Density, d_4^{25}	Index of Refraction, n_D^{25}	Specific Rotation, $[\alpha]_D^{25}$
		Pinus ayacahuite (300 Gm. Used)				
1	760	98.0	2.2	0.6841	1.3875	+ 0.00
2	20	35–45	17.7	0.7159	1.4030	+ 5.85
3	20	45–47	66.6	0.8493	1.4640	+18.00
4	10	55–60	4.0	0.8452	1.4690	−39.80
5	10	60–69	4.0	0.8452	1.4777	−15.60
6	10	69–70	1.0	0.8589	1.4793	− 3.50
7	3	70–90	2.4	0.9144	1.4865	+18.27
8	3	90–94	1.7	0.9170	1.4985	+18.52
Residue		94	0.4			
		Pinus cembroides (353 Gm. Used)				
1	17	47.5–48.5	32.9	0.8563	1.4659	+46.75
2	..	48.5–49.5	54.6	0.8558	1.4659	+45.51
3	..	49.5–50.5	1.7	0.8552	1.4659	+44.00
4	..	50.5–51.5	3.4	0.8545	1.4664	+41.70
5	..	51.5–61.5	2.8	0.8489	1.4677	+30.20
6	..	61.5–70.5	1.4	0.8438	1.4752	− 3.56
Residue	..	70.5	3.2			
		Pinus pinceana (175 Gm. Used)				
1	10	53–55	1.8	0.8452	1.4700	− 87.70
2	..	55–56	2.5	...	1.4710	...
3	..	56–57	2.8	...	1.4715	...
4	..	57–58	4.0	...	1.4716	...
5	..	58–59	3.7	...	1.4720	...
6	..	59–60	4.6	...	1.4721	...
7	..	60–61	15.4	0.8379	1.4729	−133.10
8	..	61–62	52.8	0.8379	1.4730	−134.25
9	..	62–72	1.4	...	1.4759	...
10	..	110–111	6.3	0.8973	1.4995	− 11.15
Residue and losses			4.7			

50-cc. Claissen flask. Five grams was collected at 166–170° and 5 Gm. at 170–176°. No β-pinene was found in the 166–170° fraction. The fraction which boiled at 170–176° possessed these characteristics: d_4^{25}, 0.8390; n_D^{25}, 1.4736.

Although these physical characteristics suggested the presence of limonene, bromination of this fraction in a 1:1 mixture of ether-alcohol failed to yield any crystalline bromide.

Attempts to identify Δ^3-carene in fraction 4 were also unsuccessful.

TABLE II

	Fractions 1 and 2	n-Heptane[a]
n_D	1.3867^{23}	1.3867^{23}
d	0.6746^{25}_4	0.6842^{20}_4
B. p.	98° at 764 mm.	98.52° at 760 mm.
C	83.49%	83.91%
H	16.23%	16.09%

[a] "Handbook of Chemistry and Physics," 27th ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1943, pp. 810–811.

Fraction 5 and fraction 6 redistilled over sodium possessed the physical characteristics presented in Table III; the characteristics of terpinolene are given for comparison.

Anal.—Calcd. for $C_{10}H_{16}$: C, 88.16; H, 11.84. Found: C, 87.30; H, 11.93.

When 1 Gm. of the oil of fraction 5 was dissolved in a mixture of 1 Gm. of amyl alcohol and 2 Gm. of ether and treated in an ice bath with 1 cc. of bromine, tetrabromide crystals precipitated. After three recrystallizations from chloroform (with methanol), the tetrabromide had a melting point of 115°.

Fraction 6, similarly treated, yielded more copious tetrabromide precipitate than did fraction 5. The melting point of the tetrabromide was 115.5 to 116°.

Accordingly, fraction 5 and particularly fraction 6 contained a monocyclic terpene, terpinolene. This terpene was previously found in the needle and twig oil of *Cupressus macrocarpa* (1) and in the turpentines obtained from stumps of *Pinus silvestris* (2) and of *Pinus ponderosa* (3).

Fraction 7 was a transition character and was far from pure; it reacted with metallic sodium. The microanalysis showed the following composition:

Anal.—Calcd. for $C_{10}H_{16}O$: C, 79.0; H, 10.5. Found: C, 80.94; H, 10.96.

The small quantity of the material precluded any further investigation of this fraction. Apparently it includes some oxygen-containing terpene derivatives.

Fraction 8, judging by its physical characteristics, apparently contained a sesquiterpene. A sample (3 Gm.) of the oil of this fraction dissolved in 18 cc. of absolute ether was treated with dry hydrogen chloride for thirty minutes. After standing for a week in a stoppered flask in a refrigerator, the con-

TABLE III

Material	Boiling Range, °C.	Density, d	Index of Refraction, n_D	M. P. of Tetrabromide, °C.
Fraction 5, redistilled over Na	184–186	0.8501_4^{24}	1.4809^{24}	115
Fraction 6, redistilled over Na	186–188	0.8509_4^{24}	1.4795^{24}	115.5–116
Terpinolene[a]	186–187	0.8623_4^{20}	1.4861^{20}	116
Terpinolene fraction[b]	58 at 10 mm.	0.8513_4^{20}	1.4778–1.4808	116.5–117.5

[a] Krestinski and Solodki (1).
[b] Briggs and Sutherland (2).

tents were poured into a watch glass. Ether was allowed to evaporate and the watch glass containing the oil was put back into the refrigerator. Crystals appeared in the watch glass only after a period of one month. The crystals were pressed on a porous plate and recrystallized four times from glacial acetic acid; m. p. of the chloride was 107–108°.

Judging from the molecular refraction of the oil, 65.47, fraction 8 contained a bicyclic sesquiterpene possessing two double bonds. A sesquiterpene having a dihydrochloride with a melting point of 108° has been previously reported in this series of studies[3] in turpentine of *Pinus parviflora*, which is related to *Pinus ayacahuite*.

When fraction 7 was treated similarly to fraction 8, it failed to yield a crystalline dihydrochloride.

When two drops of the oil of fraction 8 was dissolved in 2 cc. of acetic anhydride and one drop of concentrated sulfuric acid was added, the solution turned green and gradually changed to blue-green. When the flask residue was treated in the same manner, a beautiful dark green color was obtained.

To sum up, turpentine of the Mexican white pine, *Pinus ayacahuite*, from Chiapas, Mexico, consisted of *n*-heptane, 20%; *dl*-α-pinene, 70%; terpinolene, about 5%; a small amount of oxygen-containing derivatives; and a bicyclic sesquiterpene, 3%, whose dihydrochloride possesses a melting point of 108°.

PINUS CEMBROIDES

Pinus cembroides Zucc., commonly called "Mexican pinyon," grows in desert ranges in central and southeastern Arizona, southern New Mexico, western Texas, and as far south as the state of Puebla, Mexico. It also occurs in Baja California.

Oleoresin samples were collected through the courtesy of the Madero Agricultural Company of Parras, Coahuila, in a desert range called Sierra Garambullo, 5 or 6 miles from the station El Fraile of the Coahuila and Zacatecas Railroad. Local people call this pine "Pino prieto."

When the oleoresin sample arrived in Berkeley, California, the turpentine was distilled at a reduced pressure so that, at the end of the distillation, the temperature reached 170° and the pressure was reduced to 2.0 mm. of mercury. The turpentine comprised 26.2% of the weight of the oleoresin and possessed the following characteristics: d_4^{25}, 0.8471; n_D^{21}, 1.4680; $[\alpha]_D^{21}$, +45.2°. A batch of 353 Gm. of the turpentine was fractionated under 17 mm. of pressure by means of of an 80-cm. column equipped with heated jacket and a reflux head. A distillate-reflux ratio of 1:10 was maintained. The results of the fractionation are shown in Table I.

Fraction 2 was redistilled under atmospheric pressure (760.2 mm.). The boiling temperature was 155.5°; α-pinene was identified in this fraction by preparation of pinene nitrosochloride having a melting point of 103–104°, and of nitrosopiperidene, melting point 118–119°. Decrease of density as well as the boiling range of the following fractions indicated that there was no β-pinene present. The physical characteristics of fraction 6 indicated the presence of *dl*-limonene. As this fraction was optically active, perhaps small amounts of *l*-limonene were admixed there.

Fraction 6 redistilled over sodium had a boiling range of 172–176°.

After bromination of 1 Gm. of this fraction in 8 times its volume of a mixture of equal volumes of ethanol and ethyl ether, crystals of limonene tetrabromide were obtained; the crystals were pressed out on a porous plate, dissolved in a small amount of chloroform, and precipitated with methanol. Recrystallized from ethyl acetate, the tetrabromide melted at 124–125°.

The residue was redistilled at 17 mm. in a 200-cc. flask equipped with a reflux head. Most of the residue distilled over between 110 and 125°. Microanalysis showed 85.46% C; 11.47% H. Physical characteristics of this fraction were as follows: d_4^{25}, 0.9401; n_D^{25}, 1.4984; $[\alpha]_D^{25}$, +41°0′. These characteristics indicate that the fraction consisted chiefly of a dextrorotatory sesquiterpene. Three grams of the sesquiterpene was dissolved in 8 volumes of absolute ether; dry hydrogen chloride gas was passed through the ice-cooled solution for thirty minutes. The solution was then put in a glass-stoppered flask in the refrigerator. In five days, when crystals began to form in the flask, the contents were poured into a watch glass and the ether was allowed to evaporate. Hydrochloride crystals were formed in abundance. The crystals were pressed out on a porous plate and recrystallized 3 times from glacial acetic acid. The hydrochloride melted at 59°. A mixture of the hydrochloride with the longifolene hydrochloride from the Utah variety of *Pinus ponderosa* (4) showed no depression in melting point.

Accordingly, the sesquiterpene of *Pinus cembroides* was identified as *d*-longifolene, which had been originally found by Simonsen and Rau (5) in *Pinus longifolia* (*P. roxburghii*) of India.

To sum up, turpentine of *Pinus cembroides* was found to consist of *d*-α-pinene, 92%; *dl*-limonene (with a small admixture of *l*-limonene), 4%; sesquiterpene, *d*-longifolene, 2%; and polymerized residue, 2%.

[3] See THIS JOURNAL, 30, 254(1950).

PINUS PINCEANA

Pinus pinceana is a very rare pine growing in dry ravines of desert ranges in the southeastern part of the state of Coahuila, Mexico; possibly in the adjacent parts of Nuevo Leon; and (subject to verification) near Camargo, Hidalgo. Botanically, this pine at present is grouped with the pinyons, i. e., desert pines having edible nuts.

Oleoresin of *Pinus pinceana* was collected in 1950 from trees selected by the writer near the village of Garambullo, about 5 or 6 miles south of the station El Fraile of the Coahuila and Zacatecas Railroad. The oleoresin was gathered through the courtesy of the Madero Agricultural Company of Parras, Coahuila.

Oleoresin was delivered to Berkeley, California, in the fall of 1950. It was white in color with rosin acids partly crystallized, thus having a semi-solid, silky consistency and resembling in odor the oleoresin of *Pinus torreyana* (6).

A little more than 1,000 Gm. of the oleoresin was subjected to distillation under reduced pressure so that when all turpentine was removed, the temperature inside the flask was 195° and the pressure was 10 mm.; 17.7% of turpentine was obtained. The rosin was very brittle.

The turpentine had the following characteristics: d_4^{23}, 0.8434; n_D^{23}, 1.4753; $[\alpha]_D^{23}$, −100.6°.

A batch of the turpentine was distilled under reduced pressure in an 80-cm. heated-jacket Vigreux column in which a reflux-distillate ratio of 10:1 was maintained.

α-Pinene and limonene were identified in fraction 1.

The α-pinene yielded a copious precipitate of nitrosochloride which, after three recrystallizations from chloroform by means of ice-cold methanol, had a melting point of 103–104°. Fraction 1 yielded a tetrabromide after bromination of a 1-Gm. sample in 8 volumes of the 1:1 ethanol-ether mixture only after a couple of drops of water were added. The melting point of the tetrabromide was 104–105°. Fraction 8, redistilled at atmospheric pressure over sodium and with a boiling range of 176.5–177°, immediately after bromination yielded a very copious precipitate of limonene tetrabromide which, after two precipitations from chloroform by cold methanol and recrystallization from ethyl acetate, possessed a melting point of 104–105°.

Fraction 10 was redistilled at atmospheric pressure. Most of it distilled at 259–260° and possessed the following characteristics: d_4^{23}, 0.9117; n_D^{23}, 1.500. The microanalysis of the oil showed a content of 87.64% C and 11.61% H. Fraction 10 thus consisted of a sesquiterpene. The molecular refraction of the oil was found to be 65.8. One gram of the redistilled fraction 10 was dissolved in 8 volumes of absolute ether, treated with dry hydrogen chloride gas for thirty minutes, and then placed in a glass-stoppered flask in the refrigerator. In a week the contents of the flask were poured into a watch glass, and upon evaporation of ether, the remaining oil was again placed in the refrigerator; in another week the oil solidified into a crystalline mass. The crystals were pressed out on a porous clay plate and recrystallized from glacial acetic acid. After several recrystallizations from glacial acetic acid, the hydrochloride melted at 67–68°.

Judging from its molecular refraction, the sesquiterpene possesses two double bonds and is thus of a bicyclic type. No sesquiterpene of this type is known whose dihydrochloride would melt at 67–68°. Apparently this is a new sesquiterpene, not previously described in the literature. It is proposed to name this sesquiterpene *maderene*, in appreciation of the assistance rendered by the owners of the property where the oleoresin was collected.

To sum up, *Pinus pinceana* turpentine is composed of 5% *d*-α-pinene, 80% *l*-limonene, and 5% of a new sesquiterpene possessing 2 double bonds and 2 rings; its dihydrochloride has a melting point of 67–68°.

SUMMARY

Pinus ayacahuite turpentine was found to be of the following composition: 20 per cent *n*-heptane; 70 per cent *dl*-α-pinene; 5 per cent terpinolene; 3 per cent of a bicyclic sesquiterpene whose dihydrochloride possessed a melting point of 108°; and possibly a small amount (less than one per cent) of oxygenated terpene derivatives.

Pinus cembroides turpentine consists of 92 per cent *d*-α-pinene, 4 per cent *dl*-*l*-limonene, 2 per cent sesquiterpene *d*-longifolene, and 2 per cent polymerized residue.

Pinus pinceana turpentine is composed of 5 per cent *d*-α-pinene, 80 per cent *l*-limonene, and 5 per cent of a new sesquiterpene, maderene.

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Composition of Gum Turpentines of Pines. XV. A Report on *Pinus resinosa* and *Pinus reflexa*[*,†]

By N. T. MIROV

The composition of gum turpentine from two American pines has been determined. Each sample was fractionated and its components have been investigated and reported.

PINUS RESINOSA Solander is commonly called red pine or Norway pine (after the town of Norway, Maine). Its range is from Nova Scotia to northern Ontario, to southern Manitoba, southward through Massachusetts, Pennsylvania, West Virginia, central Michigan, Wisconsin, and northeastern Minnesota.

Frankforter (1) reported that steam-distilled turpentine of Norway pine possessed the following characteristics: d^{20}, 0.8636; b. p., 153–154° (presumably initial boiling point); n_D, 1.47127; $[\alpha]_D$ +17.39°. No further inquiry into the composition of the turpentine was made by him.

EXPERIMENTAL

Oleoresin used in the present experiments was obtained through the courtesy of Joseph H. Stoeckeler of U. S. Forest Service, Rhinelander, Wisc.

A batch of 1,560 Gm. of oleoresin was distilled under reduced pressure so that at the end of the distillation the temperature inside the flask reached 180° and the pressure was reduced to 4 mm. of mercury. Yield of turpentine was 20% of the weight of the oleoresin. The turpentine had these physical constants: d^{23}_4, 0.8571; n^{23}_D, 1.4668; $[\alpha]^{23}_D$, +10.00°.

A batch of 300 Gm. of turpentine was subjected to fractional distillation under reduced pressure in a flask equipped with an 80-cm. column of the Vigreaux type. The column was provided with a heated jacket and a reflux condenser. A 1:10 distillation reflux ratio was maintained. Results of the distillation are presented in Table I.

Fraction 1 was redistilled over metallic sodium at atmospheric pressure. Most of the oil distilled at 156–157°; α-pinene was identified in this fraction by the preparation of nitrosochloride having a melting point of 103–104°, and of nitrosopiperidine that melted at 118°.

Fractions 4 and 5 were examined for the presence of β-pinene by preparing sodium nopinate and nopinic acid. The nopinic acid recrystallized from benzene and melted at 126°. An admixture of known nopinic acid did not depress the melting point.

Fraction 6, redistilled over metallic sodium, had the characteristics of phellandrene: d^{24}_4, 0.8571; $n^{24.5}_D$, 1.4760; $[\alpha]^{24.5}_D$, −3.0.

When 1 Gm. of oil of the redistilled fraction 6 was mixed with 5 cc. of petroleum ether and 2 cc. of glacial acetic acid with the subsequent addition of 2 cc. of saturated solution of sodium nitrite, a precipitate of nitrosite was formed (2). Because of the small quantity of material, further purification of the nitrosite was unsuccessful and its melting point could not be determined. Therefore, although it appears that the last fraction of the *Pinus resinosa* oil contains a small amount of phellandrene, its presence (which is very interesting from a taxonomic point of view) still needs verification. The flask residue was dextrorotatory.

PINUS REFLEXA

Pinus reflexa Engelm. grows in the southwestern United States and in the adjacent parts of Mexico. The synonymy of this pine is confusing. Some botanists call it *Pinus strobiformis* Engelm.; others prefer to call it *Pinus ayacahuite* var. *brachyptera*. Still others believe that the proper name for this pine should be *Pinus flexilis* var. *reflexa*. Another group, including Dr. Maximino Martinez, calls this species *Pinus reflexa*. The present writer shares the opinion of this last group.

Samples of oleoresin of *Pinus reflexa* were collected in July and August, 1951, with the assistance of the Southwest Lumber Company, in the Apache Indian Reservation near the company's logging town of Maverick, Arizona, at an elevation of 8,100 feet.

Turpentine was separated under reduced pressure from a batch of oleoresin. At the end of the distillation, the temperature in the flask was 190° and the pressure was 3 mm. of mercury. The results of distillation were as follows:

Weight of oleoresin distilled	2,893 Gm.
Water	160 Gm.
Impurities	50 Gm.
Oil	475 Gm., or 17.7% of the pure oleoresin.

The turpentine had these characteristics: d^{24}_4, 0.8456; n^{27}_D, 1.4669; optical rotation, none.

A batch of 450 Gm. of turpentine was fractionated in a column described at the beginning of this paper.

Identification of *n*-Heptane—Fraction 1 was repeatedly shaken with fuming sulfuric acid; the unreacted part of the oil was washed with sodium carbonate and dried over anhydrous sodium sulfate. The oil was optically inactive; d^{22}_4, 0.6826; n^{25}_D, 1.3868; b. p., 98.4°. The respective values for *n*-heptane (3) are: d^{20}_4, 0.6840; n^{23}_D, 1.3867; b. p., 98.52°.

Anal.—Calcd. for C_7H_{16}: C, 83.91; H, 16.09. Found: C, 84.32; H, 15.89.

[*] Received June 3, 1952, from the California Forest and Range Experiment Station, maintained by the Forest Service, U. S. Department of Agriculture, in cooperation with the University of California, Berkeley.

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TABLE I.—RESULTS OF FRACTIONAL DISTILLATION OF PINUS RESINOSA AND OF P. REFLEXA TURPENTINES

Fractions	Pressure, Mm.	Boiling Range, ° C.	Distillate, %	Density, d^{25}_4	Index of Refraction, n^{25}_D	Specific Rotation, $[\alpha]_D$
			Pinus resinosa			
1	20	53–54	71.0	0.8534	1.4683	+12.1
2	20	54–56	5.0	0.8534	1.4693	+ 8.8
3	20	56–58	6.7	0.8534	1.4701	+ 7.0
4	20	58–60	12.3	0.8538	1.4718	+ 3.1
5	20	60–65	1.0	0.8589	1.4706	+ 2.3
6	4	47–48	2.8	0.8589	1.4781	− 3.1
Flask residue		48	1.2	0.9239	1.4985	+29.6
			Pinus reflexa			
1	765	95–145	4.2	...	...	0.0
2	765	145–150	0.3	...	1.4338	...
3	765	150–155	0.7	...	1.4553	...
4	765	155–156	70.3	0.8545	1.4694	+ 2.36
5	10	54–56	6.3	...	1.4711	...
6	10	56–60	4.5	0.8494	1.4727	+ 9.97
7	10	60–64	5.0	0.8360	1.4696	+ 5.57
8	10	64–70	0.7	0.8190	1.4637	+ 0.42
9	3	70–75	0.8	...	1.4561	...
10	3	75–80	0.6	0.8002	1.4546	− 0.82
11	3	80–90	0.5	...	1.4847	...
12	3	90–107	4.5	0.9136	1.4985	−19.00
13	3	107–130	0.4	0.9184	1.5033	−48.70
14	3	130–145	0.1	0.9065	1.5023	
Flask residue		145	1.1			

Identification of α-Pinene.—A portion of fraction 4 was redistilled over metallic sodium and treated with amyl nitrite and hydrochloric acid. Pinene nitrosochloride precipitated in abundance. Its melting point, after 4 precipitations from chloroform with methanol, was 104–105°; the melting point was not depressed by the addition of an authentic pinene nitrosochloride.

Tests for β-pinene in the redistilled fraction 5 (portions boiling between 164 and 165°) and for phellandrene in fraction 6 (b. p., 172–174°) were negative. Limonene was absent in fraction 6 and in fraction 7 (b. p., 174–178°).

Identification of Δ³-Carene.—Redistilled fraction 5 yielded 2 main fractions, possessing boiling ranges from 168 to 170° and from 170 to 172°, respectively. A nitrosate was prepared by mixing 5 Gm. of the oil with 2 Gm. of glacial acetic acid and 4 Gm. of amyl nitrite, cooled with ice, and treated with 3.5 Gm. of nitric acid. After several recrystallizations, the nitrosate melted at 142–143°; it gave no depression in melting point with Δ³-carene nitrosate obtained from *Pinus ponderosa*.[1]

Identification of Terpinolene.—Fraction 7 was redistilled over metallic sodium, and the fractions distilling at 180–185° and 185–187° (amounting together to 6 Gm.) were tested for terpinolene. A mixture of 1 Gm. of amyl alcohol, 2 Gm. of ether, and 1 Gm. of the oil was treated in the cold with bromine. After 3 precipitations from chloroform with methanol, the tetrabromide melted at 116°. An admixture of terpinolene tetrabromide prepared from the turpentine of *Pinus ayacahuite* (see Part XIV of this series) did not depress the melting point of the *Pinus reflexa* tetrabromide.

Identification of *n*-Undecane.—Combined fractions 9 and 10 were shaken repeatedly with fuming sulfuric acid, rinsed with water and 6 *N* NaOH treated with sodium bisulfite, and dried over anhydrous sodium sulfate. The unreacted, optically inactive oil had the following characteristics: d^{22}_4, 0.7397; n^{22}_D, 1.4191; boiling range at 761 mm 195–196°. The constants for *n*-undecane are: d^{20}_4, 0.741; n^{20}_D, 1.4184; b. p., 195.84° (3).

Anal.—Calcd. for $C_{11}H_{24}$: C, 84.52; H, 15.48. Found: C, 84.99; H, 15.22.

Identification of Cadinene.—Physical constants of fraction 12 indicated the presence of a sesquiterpene. This fraction was redistilled at atmospheric pressure over metallic sodium. The bulk of the fraction distilled between 256 and 258°. The oil had the following characteristics: d^{25}_4, 0.9182; n_D, 0.4983; molecular refraction, 65.5; calculated for cadinene it is 66.1. This seems to indicate that the sesquiterpene in question is of a bicyclic structure, possessing two double bonds. Three grams of the oil was dissolved in 18 cc. of absolute ether and treated in the cold with dry hydrogen chloride. The flask was kept in a refrigerator for one week and then its contents were poured in a watch glass. Hydrochloride crystals appeared on evaporation of the ether; more crystals formed after an overnight storage of the watch glass and its contents in the refrigerator. The crystals were pressed on a porous plate and after four recrystallizations they possessed a melting point of 117–118°.

Anal.—Calcd. for $C_{15}H_{24}$: C, 88.24; H, 11.77. Found: C, 87.90; H, 11.83.

Judging from the above characteristics, the sesquiterpene is cadinene.

SUMMARY

To summarize, turpentine of *Pinus resinosa* consists of *dl*- and *d*-α-pinene, 92 per cent;

[1] See THIS JOURNAL, **39**, 260(1950).

β-pinene, 3 per cent; phellandrene, 3 per cent; and perhaps about one per cent of a dextrorotatory component possessing a boiling point higher than 172°.

Turpentine of *Pinus reflexa* from Arizona has a rather complicated composition: *n*-heptane, 5 per cent; *dl*-α-pinene, 75 per cent; Δ^3-carene, 5 per cent; *d*-terpinolene 5 per cent; *n*-undecane, two to three per cent; cadinene, 5 per cent.

On the basis of chemical composition of its turpentine, it appears that *Pinus reflexa* is related to *P. monticola* (4) inasmuch as both contain *n*-undecane. On the other hand it is related to *P. ayacahuite* (5) since both species contain *n*-heptane, terpinolene, and cadinene in their turpentine. *P. flexilis* (6) does not possess any of the four above-mentioned ingredients.

REFERENCES

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(2) Guenther, E., "The Essential Oils," Vol. 2, D. Van Nostrand Co., New York, 1949, p. 42.
(3) "Handbook of Chemistry and Physics," 27th ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1947, p. 1405.
(4) Foote, P. A., and Mirov, N. T., THIS JOURNAL, **22**, 829(1933).
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Note

A Note on the Preparation of *l*-Menthyl Acetate*

By CLIFTON E. MILLER†

THE USE of isopropenyl acetate as an acetylizing agent has been described (1) with no mention, however, of its reactions with terpene alcohols. One phase of the volatile oil program in progress at this institution is the improvement of certain analytical procedures. The time-consuming method for determining menthol in peppermint oil by the use of acetic anhydride is known. The use of an acetylizing agent in which the only by-product is acetone for volatile oil acetylizations would have definite advantages. This note deals with the procedure developed for preparing *l*-menthyl acetate in yields above 90 per cent. A typical run is described herewith.

EXPERIMENTAL

Fifty-two grams of menthol (0.3 mole) was placed in a 500-cc. three-necked flask fitted with a dropping funnel, a thermometer, a 30-cm. helice-packed column arranged for distillation, and a Glas-Col heating mantle. Thirty-four grams of isopropenyl acetate[1] (0.3 mole) was added and a partial liquefication of the menthol occurred, together with a marked endothermic reaction, the average temperature drop being about 15°. The mixture was heated to 45° and 3 drops of sulfuric acid was added. The acetone formed in the reaction was continually removed by distillation, and the heating was continued to a still pot temperature of 180°.

The resulting crude menthyl acetate was then fractionated through a 60-cm. helice-packed column with a total condensation partial take-off head. The yield of ester boiling at 109–110° (10 mm.) was 62 Gm. (91% of theoretical). The following constants were determined: d_{25}^{25}, 0.9222; n_D^{25}, 1.4461; $[\alpha]_D^{25}$, −76° 19′; soluble in 4 cc. of 70% alcohol and 9 cc. of 65% alcohol.

REFERENCE

(1) Hagemeyer, H. J., Jr., and Hull, D. C., *Ind. Eng. Chem.*, **41**, 2920(1949).

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[1] Generously supplied by Carbide and Carbon Chemicals Corp.

Book Notices

Proceedings of the London Conference on Optical Instruments 1950. John Wiley and Sons, Inc., New York, 1952. xv + 264 pp. 22.5 x 14.5 cm. Price $7.00.

Optics has application in almost every field of scientific activity. Analytical chemists are particularly conscious of the advances involving applied optics that have occurred in instrumentation methods of analysis during recent years. This book, which includes papers presented during the London Conference on Optical Instruments in 1950, is composed mainly of descriptions of recent developments and improvements in optical instruments covering a wide range of applicability. Photographic and projection lenses, reflecting microscopes, gratings and grating instruments, phase-contrast microscopes, spectrophotometers, reflecting telescopes, and new optical materials were discussed during this conference through the presentation of two or three papers in each general field of interest. Summaries of discussions following their presentation are included. The book should be of especial interest to those applying optical methods to research problems and routine analyses as well as to those whose primary interests are directed toward research in optics.

Dynamic Aspects of Biochemistry. 2nd ed. By ERNEST BALDWIN. Cambridge University Press, New York, 1952. xx + 544 pp. 14 x 22 cm. Price $5.00.

The basic concepts of biochemistry have undergone much revision and enlargement in the five years since the first edition of this book was published. The new developments have been included in this revised edition, which retains the valuable features discussed in the review of the earlier edition in THIS JOURNAL, **37**, 267(1948).

The new Chapter III, on "Biological Energetics," is particularly interesting; it presents material that is important to the understanding of this elementary text and is excellent refresher material for the advanced student in the field. The clarity of presentation and the author's ability to stress the important points in this complex subject can best be illustrated by his summary of Chapter III.

The chapter on the aerobic metabolism of carbohydrates includes the citric acid cycle and the part played by coenzyme A in citrogenesis. In view of the probable identity of "active acetate" with acetylcoenzyme A rather than acetylphosphate, the chapter on fat metabolism has been entirely rewritten.

This interesting text should be available to all students and workers in the field of biochemistry.

Introduction to Mycology. By J. A. MACDONALD. Academic Press, Inc., New York, 1951. x + 177 pp. Illustrated. 14.3 x 22.3 cm. Price $3.00.

This very well-written book offers an excellent introduction to the study of mycology. The study of the fungi, often only with regard to their edibility, goes back to Roman times. Dr. Macdonald brings this study up to the present, and he prepares the student for more intensive research into past reports and new investigations.

The text is a simplified arrangement of material that is presented in a clear and unencumbered manner. *Penicillium notatum* Westling and the production of penicillin are allotted five lines, while ergot is discussed more fully in ten lines. Plant diseases and their prevention are properly discussed at length.

Many clear and fully explained illustrations are very helpful. A bibliography and a general index are appended. The book is an excellent text, and should be available to all students.

BOOKS RECEIVED

Antibiotics—A Survey of Their Properties and Uses. Published by direction of the Council of the Pharmaceutical Society of Great Britain. The Pharmaceutical Press, London, 1952. xii + 290 pp. Illustrated. 23 x 15 cm. Price 25s. (Postage 10d.)

Introductory Mycology. By CONSTANTINE JOHN ALEXOPOULOS. John Wiley & Sons, Inc., New York, 1952. Illustrated. xiii + 482 pp. 23.5 x 15.5 cm. Price $7.00.

Plants of the Bible. By H. N. and A. L. MOLDENKE. The Chronica Botanica Co., Waltham, Mass.; Stechert-Hafner, Inc., New York; 1952. Illustrated. xx + 328 pp. 18 x 27 cm. Price $7.50.

The Extra Pharmacopoeia (Martindale). Vol. I, 23rd ed. Published by direction of the Council of the Pharmaceutical Society of Great Britain. The Pharmaceutical Press, London, 1952. xxii + 1352 pp. 13 x 19 cm. Price 55s; postage 1s.

The Common and Systematic Nomenclature of the Simpler Organic Compounds. By FRED SEMENIUK. North Carolina Pharmaceutical Research Foundation, Inc., Chapel Hill, 1952. 55 pp. 28 x 21.5 cm. Price $1.25.

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